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HYPERTENSIVE CARDIOVASCULAR DISEASE ("MALIGNANT HYPERTENSION")

Changes in Canine Tissues Induced by Various Manipulations of the Kidney, with Special Reference to Vascular and Myocardial Lesions

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IN PREVIOUS communications[1] we have shown that bilateral nephrectomy induces in the dog an elevation of blood pressure and tissue changes comparable to those observed in "malignant hypertension." On the other hand, in the presence of normal renal tissue in the body, as when one ureter is implanted into the duodenum or into the vena cava or when one ureter is ligated and the contralateral kidney removed at a subsequent date, no sustained elevation of blood pressure consistently ensues.

There has been much discussion concerning the genesis of the arterial and arteriolar necrosis observed in man and in the experimental animal dying of "malignant hypertension." Some[2] have considered these changes to be the result of the elevation in blood pressure; others attribute them to at least two factors, namely, the elevation of blood pressure and excretory renal failure.[3] The present study attempts to throw light on the subject by an examination of the pathologic changes observed under varying conditions in which the blood pressure remained normal in some cases while in others it was elevated. Renal tissue was present in the organisms in certain experiments and absent in others. In addition to the study of the effects of bilateral nephrectomy, the implanting of a ureter into the bowel or the vena cava and the ligature of the ureters, we have studied the effects of cutting the ureters and allowing the urine

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1. (*a*) Grollman, A.; Muirhead, E. E., and Vanatta, J.: Am. J. Physiol. **157**:21, 1949. (*b*) Muirhead, E. E.; Vanatta, J., and Grollman, A.: Arch. Path. **48**:234, 1949.

2. Byrom, F. B., and Dodson, L. F.: J. Path. & Bact. **60**:357, 1948.

3. Goldblatt, H.: Physiol. Rev. **27**:120, 1947.

to accumulate in the peritoneal cavity and the effects of intraperitoneal injections of a sterile concentrate of dialyzed urine.

METHODS

The methods employed for performing the various surgical operations (nephrectomy, ureteral ligation, implanting of a ureter into the bowel, etc.) were similar to those reported previously,[1a] as were also the dietary measures and the using of the artificial kidney [4] to prolong the life of the animals. In the experiments in which the ureters were sectioned, these structures were approached transperitoneally, ligated near the bladder and cut across distal to the ligature. The renal circulation presumably was not interfered with except as it might be affected by reflex changes. In the experiments in which urine was injected, this was collected from normal dogs by catheterization, concentrated to one fifth of its volume by vacuum distillation at 40 C. and filtered through a Seitz filter. These concentrates were injected intraperitoneally twice daily in doses of 25 cc.

Exchange transfusion, advocated by Bessis,[5] was conducted by transfusing three to six times the estimated volume of the recipient's blood, obtained from healthy donor dogs. This blood was heparinized and administered through the femoral vein at a rate of approximately 6 cc. per minute. After 50 cc. had been infused, an equal amount was removed from the femoral artery of the recipient. The entire exchange required approximately three hours. In this way the volume of the cardiovascular system was never altered more than 50 cc. from the normal.

Peritoneal lavage was performed by the procedure of Frank, Seligman and Fine [6] except that the fluid used for the irrigation had the same composition as the bath fluid which we had found to be most suitable for use in the artificial kidney.[4]

The animals either were killed for autopsy or, if dead of disease, were examined as soon after death as possible. Sections were taken from tissues with gross lesions and also by random sampling, fixed in formaldehyde solution U. S. P. diluted 1:10 and prepared by the routine hematoxylin and eosin staining technic.

RESULTS

The lesions observed in these studies were of the same type as those described in our previous communication.[1b] In general, the basic lesions encountered can be divided into two main groups: (1) those observed in circulatory failure, including generalized venocapillary hyperemia, visceral edema, pulmonary edema and capillary hemorrhages, centrolobular damage of the liver, edema and microscopic hemorrhages of the brain and leukocytic infiltration of the adrenal cortex; and (2) lesions of muscular tissue reflected mainly by degeneration and necrosis of the heart, the arteries and the arterioles. The necrosis of the latter suggests morphologically that observed in what is called malignant hypertension or malignant arteriolar nephrosclerosis in the human patient and which is generally considered as an essential component of this disorder.[1b]

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4. Vanatta, J.; Muirhead, E. E., and Grollman, A.: Am. J. Physiol. **156**:443, 1949.
5. Bessis, M.: Blood **4**:324, 1949.
6. Frank, H. A.; Seligman, A. M., and Fine, J.: J. A. M. A. **130**:703, 1946.

The numbers of animals in which the various types of tissue changes were encountered are recorded in the table. The groups will be described separately. It must be emphasized that the data in the table indicate the frequency of lesions observed grossly and by random

Incidence of Each Type of Lesion Encountered in Dogs Which Had Undergone Various Manipulations of the Kidney and Interferences with Renal Excretory Function

	Both Kidneys Removed and Dogs Treated by:				Both Ureters Ligated, or One Ligated and Contralateral Kidney Removed	One Ureter Implanted into Intestine; Contralateral Kidney Removed	One Ureter Implanted into Vena Cava; Contralateral Kidney Removed or Left	Ureters Sectioned and Urine Drained Intraperitoneally	Urine Injected Intraperitoneally (Kidneys Intact)
	Diet	Artificial Kidney	Exchange Transfusion	Peritoneal Lavage					
Animals	12	42	2	6	21	7	21	4	8
Heart:									
Subendocardial hemorrhage	5	22	2	2	14	2	5	3	1
Myocardial hemorrhage	4	19	2	1	9	4	4	2	1
Infarct-like necrosis	2	9	1	2	12	3	3	3	2
Myocardial degeneration, focal	1	4	2	3	4	3	4	2	4
Necrosis of blood vessels	4	18	1	0	15	1	3	4	1
Lungs:									
Hyperemia, edema, capillary hemorrhages	9	32	2	2	16	4	10	4	4
Confluent hemorrhages	2	2	2	0	5	3	5	2	1
Hyaline emboli	2	13	0	0	9	1	1	0	0
Bronchopneumonia	3	4	0	0	1	1	5	0	2
Stomach:									
Necrosis of blood vessels	0	5	1	2	3	0	1	0	1
Hemorrhage	1	13	0	0	2	0	3	0	0
Focal necrosis of mucosa	1	6	1	1	1	0	2	0	0
Intestine:									
Necrosis of blood vessels	0	10	2	2	5	0	1	0	0
Hemorrhage	0	1	0	1	0	1	4	0	0
Focal necrosis	0	4	1	2	1	1	1	1	0
Swelling of smooth muscle	10	22	2	0	13	7	5	4	2
Liver:									
Hyperemia	5	22	2	0	11	2	7	3	2
Centrolobular damage	3	16	1	0	7	1	3	2	0
Necrosis of blood vessels	1	1	0	0	2	0	0	1	0
Spleen:									
Hemosiderosis	0	5	1	4	3	4	2	0	2
Pancreas:									
Necrosis of blood vessels	2	4	0	0	0	0	0	0	0
Adrenal:									
Necrosis of blood vessels	2	5	0	0	3	0	0	0	0
Leukocytic infiltration	0	3	0	0	3	1	0	1	0
Urinary bladder:									
Swelling of smooth muscle	0	2	1	0	2	1	5	3	1
Necrosis of blood vessels	0	0	1	0	1	0	0	0	0
Skeletal muscle:									
Degeneration	0	6	1	0	2	2	1	1	0
Brain:									
Hyperemia, edema and capillary hemorrhage	6	10	2	0	9	2	4	4	2

sampling; examination of serial sections would most likely have revealed a greater incidence of each type of lesion.

GROUP 1. *Both Kidneys Removed.*—In the first column of the table are shown the results of bilateral nephrectomy observed in animals which had been maintained alive for at least five days by dietary measures alone without being subjected to artificial measures for the removal of waste products. In the second, third and

fourth columns are shown the results obtained in nephrectomized animals when use was made of the artificial kidney, exchange blood transfusion and peritoneal irrigation, respectively. The animals to which the artificial kidney was applied survived for a period of six to nineteen and one-half days; the 2 animals subjected to exchange transfusions died five and six days after nephrectomy, while those subjected to peritoneal irrigation were killed on the seventh to twelfth day. In the last two groups of dogs the blood pressure rose to hypertensive levels similar

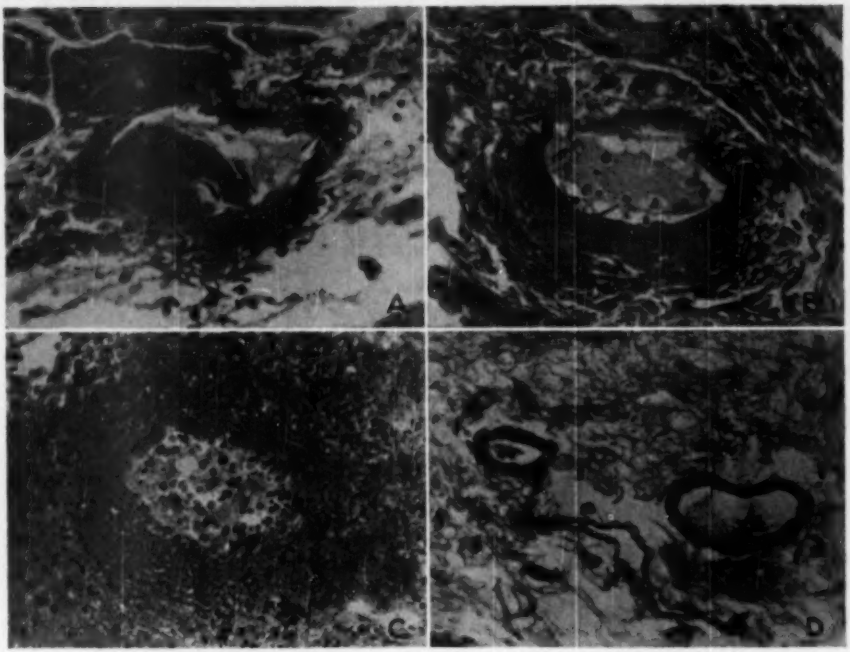

Fig. 1.—Necrosis of blood vessels. *A* (dog 180; survival, six days following bilateral nephrectomy and exchange transfusion; blood pressure, 180 mm. of mercury), coronary arterial branch in heart, showing necrotic media with smudging toward adventitia, intact endothelium and a subendothelial granular mass. × 235.

B (same dog as in *A*), artery of submucosa of the intestine with necrotic media containing small vacuoles. Muscle fibers in the upper half of the media are intact. × 245.5.

C (same dog as in *A*) blood vessel in the edematous submucosa of the stomach with necrotic media and polymorphonuclear neutrophilic leukocytes and lymphocytes in the adventitia. This lesion, resembling periarteritis nodosa, has been infrequently encountered, usually in the submucosa of the gastrointestinal tract. × 256.

D (dog 185; killed on seventh day following bilateral nephrectomy and peritoneal irrigation; blood pressure, 170 mm. of mercury), arterial branches in the submucosa of the stomach, showing necrosis of the media and intact endothelium. The vessel on the right is partly hyaline in appearance, while the other vessel reveals granular necrosis of the media. × 276.

to those observed when nephrectomized animals were maintained with the artificial kidney or by dietary measures alone.[1a]

Comment.—As noted from the table, there is no marked difference in the nature and the incidence of the lesions regardless of whether dietary measures alone, the artificial kidney, blood exchange transfusion or peritoneal lavage is used to prolong survival, a fact indicating that the procedures utilized are not responsible for their occurrence. Moreover, the hypertensive dogs that were killed prior to the onset of the terminal circulatory failure revealed lesions of the heart and blood vessels (figs. 1, 3 and 4), thus demonstrating that these lesions are not dependent on circulatory failure for their development.

Group 2. *Both Ureters Ligated or One Ligated and the Contralateral Kidney Removed.*—In column 5 of the table is shown the incidence of each type of lesion in 21 dogs subjected to bilateral ligature of the ureters or unilateral ligature with removal of the contralateral kidney. This operation may induce papillary necrosis and tubular degeneration of the kidney,[7] as well as a transient rise in blood pressure.[1a] Impressive lesions occurred in these animals, whether aided with the artificial kidney or not. In all the animals the lesions observed were similar to those following bilateral nephrectomy, with changes due to circulatory failure being prominent. The heart was particularly affected by subendocardial hemorrhages, areas of "infarct-like" necrosis, and necrosis of branches of the coronary arteries (figs. 2 and 3). Other vessels, particularly those of the gastrointestinal tract, likewise were the seat of medial necrosis and smudging.

Group 3. *One Ureter Implanted into the Small Intestine; Contralateral Kidney Removed.*—In the 7 dogs comprising this group, the ureter which had been implanted into the small bowel was found at autopsy to be patent. As shown previously,[1a] the reabsorption of the urine draining into the upper bowel of the dog results in abnormalities of the body fluids comparable to those observed following bilateral nephrectomy. Arterial hypertension was not observed in this group except in 1 animal in which the mean blood pressure reached levels of 150 to 160 mm. of mercury, compared with 120 mm. prior to the operation.

Of the 7 dogs in this series, 5 were treated with the artificial kidney. Of these

(*a*) One animal, to which the artificial kidney had been applied on six occasions, was killed on the thirtieth day following the contralateral nephrectomy. All its tissues appeared normal.

(*b*) One animal died on the seventeenth day following nephrectomy after five periods of dialysis. It also had no cardiac or vascular lesions, but there was focal suppurative pyelonephritis and hemosiderosis of the extremely hydronephrotic remaining kidney. There was also extensive calcification of the aorta, the pulmonary arteries and the diaphragm, which may have been present prior to the experiment. Focal necrosis was noted in the smooth muscle of the bowel and in the skeletal muscle.

(*c*) Two animals died on the sixth day after being subjected to one period of dialysis on the fourth and fifth days, respectively. The remaining kidney of each animal was normal in appearance, but the heart showed evidence of extensive damage (necrosis and granular degeneration), and there was evidence of circulatory failure. One of these animals also had necrosis of branches of the coronary artery.

(*d*) The fifth animal of this group was subjected to two periods of dialysis and was killed on the twelfth day following nephrectomy. The remaining kidney

7. Muirhead, E. E.; Grollman, A., and Vanatta, J.: J. A. M. A. **142**:627, 1950.

revealed hydronephrosis, papillary necrosis, dilatation and atrophy of the tubules and hemosiderosis. The heart displayed subendocardial and focal myocardial hemorrhages, focal granular and hyaline degeneration of myocardial fibers and infarct-like areas of necrosis. Necrotic blood vessels were not identified in the sections examined.

(*c*) The 2 remaining animals of this series which were not treated by dialysis died on the twelfth day following the contralateral nephrectomy. At autopsy both animals showed evidence of circulatory failure, swelling of the smooth muscle of the intestinal tract and of the urinary bladder, hemosiderosis of the spleen and moderate hydronephrosis of the remaining kidney with compression of the tips of the papillae (papillary necrosis). One of these animals displayed in addition focal hemorrhages and granular and hyaline degeneration of the myocardium.

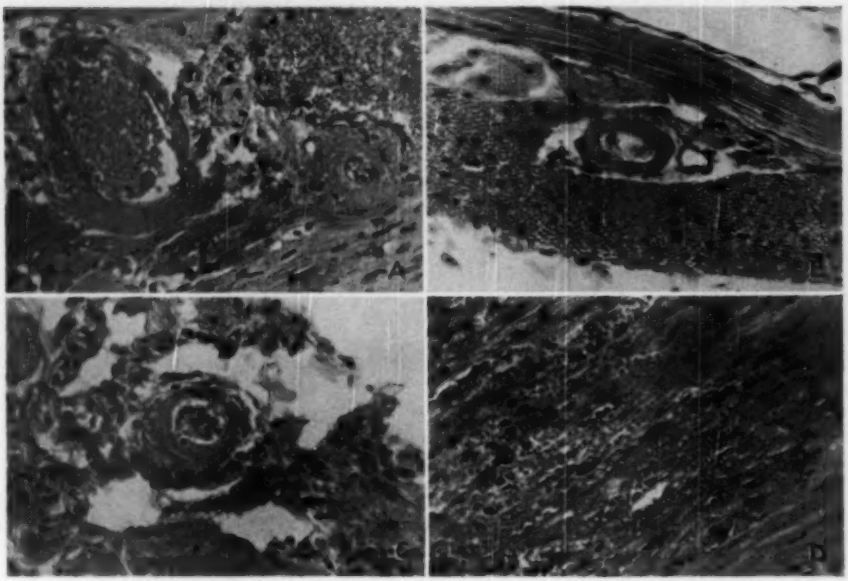

Fig. 2.—Necrosis of blood vessels. *A* (dog 179; killed ten days after unilateral ureteral ligation and contralateral nephrectomy; blood pressure, 180 mm. of mercury), two necrotic vessels in the myocardium and regional hemorrhage. The larger vessel on the left shows lumpy granular disintegration of the media, pyknosis of the nuclei and smudging of the lower portion of the media. × 211.

B (same dog as in *A*), a vessel of arteriolar size in the muscularis of the small intestine, showing necrosis and early hyaline change of the media. × 369.5.

C (dog 150; died on tenth day following start of daily intraperitoneal injections of concentrate of urine; blood pressure, 100 mm. of mercury), necrosis of gastric blood vessel. This animal also revealed marked degeneration of renal tubules. × 224.5.

D (dog M 1; killed on thirteenth day after bilateral nephrectomy with peritoneal irrigation in the interim; blood pressure, 180 mm. of mercury), focal necrosis and hemorrhage of muscularis of small intestine with hyaline swelling and fibrinoid necrosis of muscle fibers. × 237.5.

An eighth animal, although excluded from this series, may be discussed here. After it had undergone one period of dialysis, its blood urea level failed to rise subsequently above 300 mg. per hundred cubic centimeters. At autopsy we found that the ureteral implantation had been made in the large bowel 10 cm. above the rectal sphincter. This animal manifested a prolonged state of nitrogenous retention with a blood urea level averaging 260 mg. per hundred cubic centimeters. The animal appeared healthy and was killed after eighteen days. Autopsy revealed normal-appearing tissues. Throughout this period the blood pressure was normal.

Comment.—Elevation of the blood pressure occurred in only 1 of these 7 dogs, and this animal showed evidence of renal damage (papillary necrosis and tubular

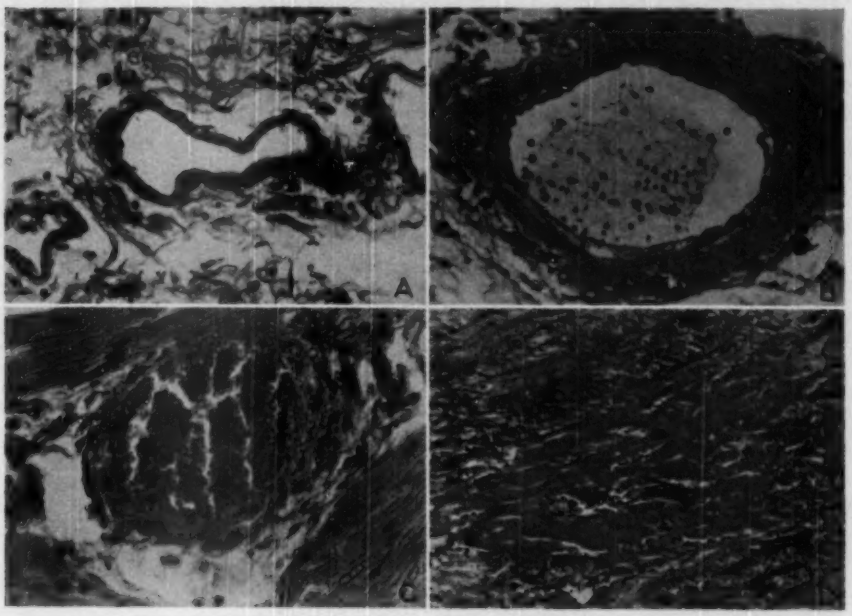

Fig. 3.—Vascular and myocardial necrosis. *A* (dog 118; died on seventh day after ureter was implanted into vena cava; blood pressure, 120 mm. of mercury), vessel in edematous submucosa of stomach, showing necrosis of its media; pyknotic nuclear remnants remain. × 244.5.

B (same dog as in fig. 2*A*), intrarenal artery of the remaining hydronephrotic kidney, showing necrosis of the media with smudging and the presence of vacuoles. This is a rare finding in this series. × 410.5.

C (dog 133; died on third day after its ureters were cut, with urine draining into the peritoneal cavity; blood pressure, 90 mm. of mercury), necrotizing artery of myocardium showing lumpy and granular disintegration of smooth muscle with smudging of media. × 254.

D (dog 36; died on third day after bilateral ureteral ligation; blood pressure, 135 mm. of mercury), necrosis of myocardium with regional hemorrhage and scattered neutrophils. × 122.

degeneration). It is apparent that lesions may occur in the absence of arterial hypertension. However, in the 2 animals which survived for prolonged periods (thirty and seventeen days) through the use of the artificial kidney, no vascular or myocardial lesions were encountered. The remaining 5 dogs displayed varying degrees of vascular and myocardial necrosis, with gross renal lesions in 3.

GROUP 4. *One Ureter Implanted into the Vena Cava; Contralateral Kidney Removed or Left.*—Of the 11 animals of the series which had a patent anastomosis, 10 remained normotensive throughout; in 1 the blood pressure rose to 150 mm. of mercury, compared with the preoperative level of 130 mm.

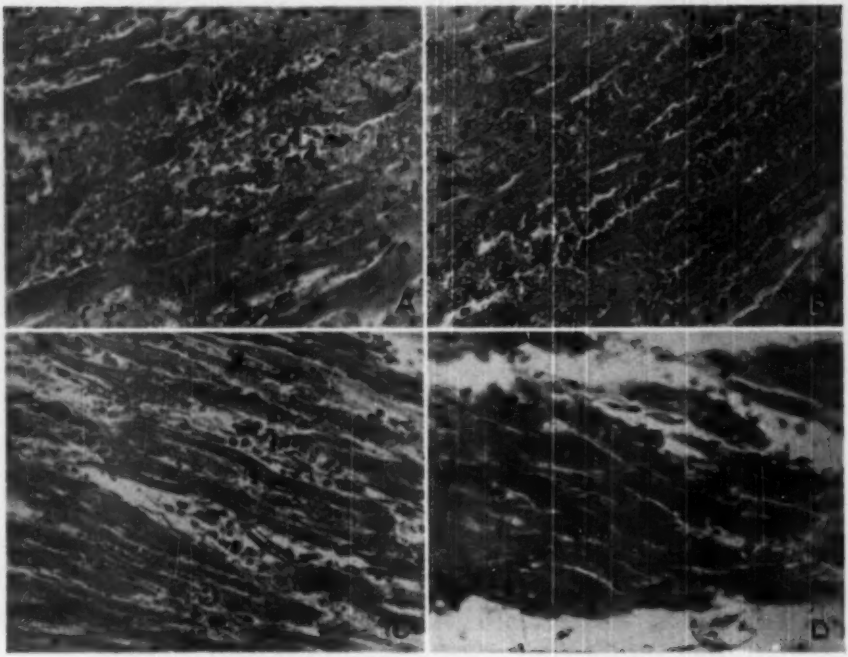

Fig. 4.—Necrosis of myocardium. *A* (same dog as in fig. 1*A*, with bilateral nephrectomy and exchange transfusion), focal necrosis, lumpy and granular disintegration of myocardium and hemorrhage. × 256.

B (same dog as in fig. 2*A*, with unilateral ureteral ligation and contralateral nephrectomy), focal necrosis and hemorrhage of myocardium. × 256.

C (dog 118; died on seventh day after ureter was implanted into vena cava and contralateral kidney removed; blood pressure, 150 mm. of mercury), granular and hyaline degeneration and necrosis of myocardium. × 256.

D (dog 148; killed on fifth day after start of daily intraperitoneal injections of concentrate of urine; blood pressure, 95 mm. of mercury), focal granular and hyaline degeneration of myocardium. × 245.5.

(*a*) In 3 dogs the contralateral kidney was not removed, and they were not subjected to dialysis. Of these, 1 was killed on the twenty-sixth day and showed

only slight chronic pyelonephritis. One animal died twelve days postoperatively and revealed necrosis of the branches of the coronary arteries. The kidneys were unaltered morphologically. The third animal survived thirteen days and revealed hemorrhage and medial necrosis of the vena cava at the site of the ureteral implant, as well as widespread necrosis of coronary arterial branches and necrosis and hemorrhage of the heart. The kidneys were intensely hyperemic but were intact except for slight focal pyelonephritis of the kidney attached to the vena cava.

(*b*) The remaining 8 animals had the contralateral kidney removed; 6 were subjected to the artificial kidney once but survived only four to eight days. (1) In 2 of the 8 dogs the remaining kidney was intact and essentially normal. Evidence of circulatory failure was observed in both. In one there was intense hyperemia of the intestinal mucosa and swelling of the intestinal smooth muscle. In the other, in addition to these changes, there were hemorrhages in the intestinal muscles and widespread necrosis of the arteries, and hemorrhages, degeneration and necrosis of the myocardium (fig. 4). (2) Four animals had hydronephrosis with evidence of renal damage as manifested by compression of the parenchyma, papillary necrosis and definite degeneration and necrosis of the tubules. Suppurative pyelonephritis was noted in 2 and multiple cortical hemorrhages in 1. These animals survived for three, four, five and eight days, respectively. One of them died with intussusception of the bowel; 3 revealed hemorrhagic bronchopneumonia. All 4 showed hemorrhage, degeneration and necrosis of the heart and the gastrointestinal tract. In only 1 was there necrosis of arterial branches, and this occurred in the remaining kidney. One animal, in addition, had diffuse areas of necrosis with and without suppuration throughout the viscera. (3) The 2 remaining animals in this group (4*b*) were not subjected to dialysis and died three and four days, respectively, after contralateral nephrectomy. One succumbed to intussusception and the other to bronchopneumonia. Neither animal revealed vascular or myocardial lesions.

Comment.—This group also displayed in all but 1 instance "uremia" without arterial hypertension. Lesions of the myocardium and blood vessels developed even when the intact contralateral kidney appeared normal grossly and microscopically.

The group with contralateral nephrectomy likewise yielded vascular and myocardial necrosis with and without the presence of evident damage to the remaining kidney.

It is of interest that 5 of the 11 dogs of this series revealed confluent pulmonary hemorrhages giving rise to a hemorrhagic consolidation. These were not infarcted areas, as demonstrated by the absence of necrosis and emboli. Urine that has been diverted into the blood appears to act as an endothelial toxin, as evidenced also by the finding of hemorrhages and medial necrosis of the vena cava at the site of the ureteral implant in 3 animals.

GROUP 5. *Ureters Sectioned and Urine Drained into the Peritoneal Cavity.*—The 4 animals of this group survived in poor condition for one and a half to three days. None of them manifested arterial hypertension. At autopsy, extensive damage was noted, and there was evidence of heart failure. The heart revealed hemorrhages, infarct-like areas of necrosis, granular degeneration (fig. 5) and arterial necrosis. Extensive edema and capillary hemorrhages of the lungs, swelling of the smooth muscle of the gastrointestinal tract and necrosis of blood vessels were also noted.

GROUP 6. *Kidneys Left Intact and Concentrated Dialyzed Urine Injected Intraperitoneally.*—Hypertension developed in none of the 8 animals of this group. The following tissue changes were noted:

(*a*) Two animals, one killed after five days and the other dying on the tenth day, showed necrosis, degeneration and hemorrhages of the myocardium. One of these animals revealed in addition arterial necrosis (fig. 2) in the myocardium and the stomach. Evidence of circulatory failure was noted in both. The kidneys showed degeneration of the tubules, particularly of the proximal segment.

(*b*) Four animals, 2 killed on the eighth and eighteenth days, and 2 dying on the second and ninth days, revealed focal granular and hyaline degeneration of the myocardium. Swelling of the intestinal smooth muscle was noted in 3 of the animals. The kidneys of 3 were morphologically unaltered while that of the fourth revealed marked tubular degeneration, particularly of the proximal segment.

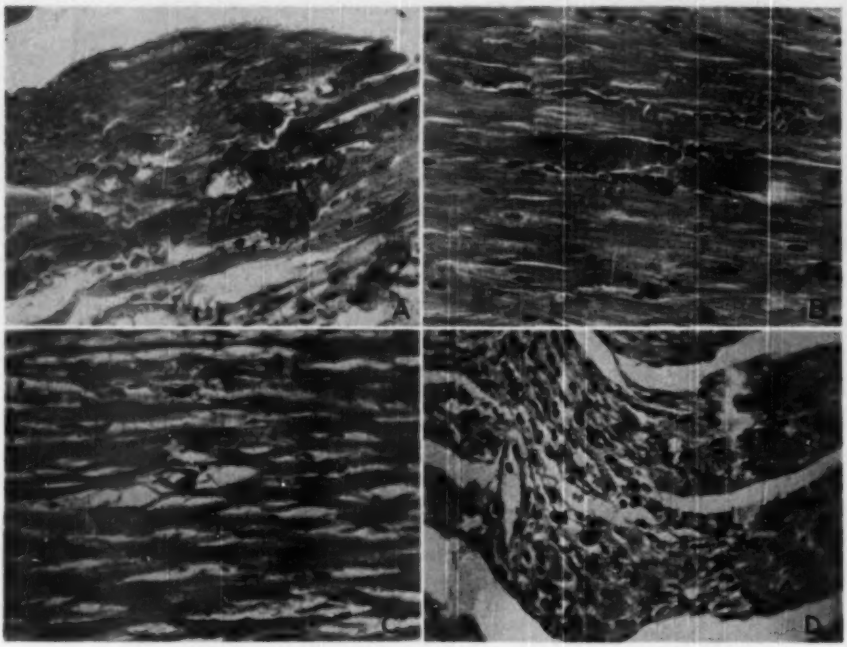

Fig. 5.—Necrosis of myocardium. *A* (dog 133; died three days after ureters were cut, with urine draining into peritoneal cavity), focal disintegration of myocardial fibers. Note the lumpy and hyaline spread of the fibers. Four parallel fibers with their syncytial connections are involved. The nuclei in this zone are pyknotic. × 266.

B (same dog as in fig. 2*D*, with bilateral nephrectomy and peritoneal irrigation), isolated necrosis of a myocardial fiber with granular disintegration and mobilization of polymorphonuclear neutrophilic leukocytes. × 276.

C (same dog as in *B*), granular and hyaline necrosis of myocardium involving the subendocardial zone of the left ventricle. No necrotic blood vessels were seen over a large microscopic area showing this form of involvement. × 276.

D (same dog as in *B*), zone of proliferation of connective tissue cells and accumulation of macrophages in an area of necrosis similar to that depicted in *C*. × 256.

(*c*) The remaining 2 animals were killed on the seventh and ninth days, respectively, and showed normal appearing tissues.

Comment.—This group likewise yielded vascular and myocardial lesions (fig. 3) both in the presence and in the absence of demonstrable renal damage.

COMMENT

The results of the present study indicate that an elevation of the arterial blood pressure is not essential for the development of arterial and arteriolar necrosis similar to that occurring in "malignant hypertension," or malignant nephrosclerosis. Following bilateral nephrectomy and the extended survival of the animals, arterial hypertension and arterial and arteriolar necrosis occurred. However, the other types of renal manipulations and interferences induced an identical form of vascular necrosis without concomitant hypertension.

The fact that the arterial and arteriolar necrosis occurs in the absence of kidneys (group 1) or when the kidneys are altered by hydronephrosis (group 2) incriminates the kidneys in the genesis of the vascular lesions. In groups 3 to 6, however, the arterial necrosis was observed in the absence as well as in the presence of morphologic renal changes evident by gross and microscopic examination. If one attributes the observed changes to renal disturbance, one must assume, therefore, that the functional disturbance of the kidneys responsible for the arterial and arteriolar necrosis can occur even though morphologically the kidneys appear intact.

Some have considered uremia as the main factor in the genesis of the vascular necrosis observed in "malignant hypertension."[3] In all the present experiments renal excretory failure existed so far as the normal excretion of urine and the waste products accumulating in the body fluids were concerned. However, the various procedures used to remove waste products from the body prevented the development of prolonged and extensive grades of uremia. With an average moderate nitrogenous retention, survival was prolonged, but vascular necrosis nevertheless developed.

Three animals surviving more than fifteen days with an elevation in nitrogenous waste products equal to that observed in the bilaterally nephrectomized dogs and those with ureteral ligations failed to reveal the vascular necrosis. In these 3 instances the remaining kidney had its ureter attached to the bowel. Since survival beyond ten days following bilateral nephrectomy or ureteral ligation has evoked vascular necrosis, it appears that moderate uremia per se is not the factor causing the vascular lesions. One might consider the accumulation of an angiotoxic waste product which fails to pass through the cellophane or peritoneal membrane during irrigation, but this is unlikely since vascular

necrosis was observed following daily massive exchange transfusions when no membrane was involved.

Although a certain degree of species difference exists between man and the dog, the type of necrosis of the small arteries and arterioles associated with "malignant hypertension" is quite similar in the two species.[1b] In man one also encounters evidence against the role of uremia alone as the cause of the vascular necrosis, for in so-called lower nephron nephrosis a prominent grade of acute uremia may exist for one to three weeks without the concomitant developments of necrosis of small arteries and arterioles. In the one patient of our series who at autopsy revealed vascular necrosis, hypertension of severe degree was present prior to death.

The fact that vascular necrosis was induced by the injection of urinary concentrates would appear offhand to favor the view that some waste product excreted by the kidney is responsible for the lesion. However, in these experiments degeneration of renal tubules was noted in the 3 animals revealing a prominent degree of myocardial and vascular necrosis. One may, therefore, attribute the presence of these lesions to renal damage rather than to the presence of a hypothetic catabolite. The fact that the lesions occur in the absence of all renal tissue indicates that they cannot be due to some toxin arising from damaged renal tissue as postulated by Winternitz and co-workers.[8]

Vascular and intestinal necrosis comparable to those which we have observed were described by Leiter and Eichelberger,[9] who injected renal extracts into dogs after constriction of the renal artery. However, their conclusion that "functioning kidney may have an important bearing on the development of hemorrhagic necrosis" is obviously untenable since we have obtained these lesions in the nephrectomized animal.

The recent experiments of Byrom and Dodson [2] in which massive volumes of Ringer's solution were infused into the arteries, with resultant damage to the blood vessels, may be accounted for by assuming that the kidneys were also damaged by this manipulation. This is rendered probable by the fact that three days were required before the vascular necrosis appeared, which would favor the view that the kidney played an intermediate role in its production. We have been unable to confirm these experiments in the dog by rapidly infusing compatible blood into the renal artery.

Myocardial necrosis, which was frequently extensive, was a striking feature of the disease produced in our experiments. This necrosis

8. Winternitz, M. C.; Mylon, E., and Katzenstein, R.: Yale J. Biol. & Med. **13**:595, 1941.

9. Leiter, L., and Eichelberger, L.: J. Mt. Sinai Hosp. **8**:744, 1942.

involved mainly the subendocardial zone of the left ventricle near the apex and the papillary muscles and was usually associated with subendocardial and myocardial hemorrhages (figs. 2, 4 and 5). Although vascular necrosis of the coronary vessels was frequently also present, we do not feel that the myocardial necrosis was entirely secondary to those vascular lesions since they occurred at times in the absence of any demonstrable regional vascular lesions, were focal in distribution and frequently consisted in the disintegration of isolated segments of single fibers. The lesions resemble those of the myocardial necrosis described in the uremic state.[10] They were encountered when the animals were killed before the development of peripheral circulatory failure and hence cannot be attributed to the development of shock.

The hyaline swellings in the smooth muscle of the viscera and skeletal muscle were similar to those described previously.[1b] In certain instances the swollen fibers resembled the so-called contraction bands, which are generally considered as artefacts. Another type of swelling which appears to be of more significance consists of individual fibers with oval or round hyaline masses within their sarcoplasm. Such areas are at times infiltrated with polymorphonuclear neutrophilic leukocytes. Intussusception of the bowel occurred in 11 per cent of the present series and was associated with these changes in the smooth muscle of the intestinal tract.

It is noteworthy that vascular necrosis did not occur in the lungs, nor were changes observed in the smooth muscle of the bronchial tree, which is in keeping with the findings in the human patient dying of "malignant hypertension."

McCormick and Holman [11] have also observed necrotizing arteries similar to those described in the present study, as a result of feeding their dogs a high fat diet and subsequently producing renal damage in the animals. Although cognizant of the importance of the derangement of renal function and of the minor role of azotemia (since ureteral ligation in their experiments failed to induce the lesions), these authors stressed the importance of diet in the causation of the lesions. However, since similar lesions are produced by nephrectomy alone, one need not consider a high fat diet as essential for the development of the vascular necrosis.

SUMMARY

The pathologic changes induced in the dog by ligation of the ureters, implanting the ureter into the bowel or vena cava, cutting the ureters,

10. Smadel, J. E., and Farr, L. E.: Am. J. Path. **15**:199, 1939. Solomon, C.; Roberts, J. E., and Lisa, V. R.: ibid. **18**:729, 1942.

11. McCormick, J. H., and Holman, R. L.: Proc. Soc. Exper. Biol. & Med. **72**:75, 1949.

and by injecting concentrated dialyzed urine intraperitoneally were noted in an effort to determine the genesis of the lesions observed in "malignant hypertension." The observations indicate that the vascular necrosis of noninflammatory nature and the degeneration and necrosis of the myocardium characteristic of "malignant hypertension" in the dog are due neither to the elevation in blood pressure nor to the accumulation of the usual catabolites to which these changes have previously been attributed.

CRYSTALLINE ESTER CHOLESTEROL AND ADULT CORTICAL RENAL TUMORS

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AN IMPORTANT medical principle is that disturbances in the supply or the quality or in the metabolism of the essential substances of the diet may give rise to disease. From this point of view it would be indeed remarkable if modification of the supply or faults of the metabolism of so important a substance as cholesterol should not give rise to disease. It is true, as Schoenheimer demonstrated, that the amount of this substance synthesized in the body rather than the quantity ingested determines the cholesterol balance in the experimental mouse. The reaction of the rabbit (herbivorous) to cholesterol feeding does not support this thesis. Moreover, among mammals atherosclerosis is an almost exclusively human disease. The evidence from autopsy studies in Germany during the fat famine in the latter part of, and following the end of, World War I demonstrated, according to Aschoff,[1] that the lack of fats was associated with a lowering of the frequency and the degree of human atherosclerotic lesions. This appears to be more dependable evidence with reference to the relation of dietary cholesterol and the human disease than that obtained from studies of the metabolism of this substance in the mouse.

Gainsboro[2] treated a considerable series of patients for glomerulonephritis and lipoid nephrosis with different feedings over long periods. He concluded that the level of the plasma content of ester cholesterol was much more dependent on the intake of sterol in the food than was that of the free cholesterol.

Studies under polarized light reveal that fresh frozen sections of human or animal tissues are likely to show only two substances with doubly refringent crystals (cholesterol and fat crystals) save in relation to new growths. Visible cholesterol occurs in the form of crystalline esters or in solid rhomboid plates. The crystalline esters—the fluid crystals of Lehmann—are spheroidal droplets or drops of a semifluid greaselike material, with Maltese cross markings under polarized light. The second type—fat crystals—takes the form of needle crystals, fre-

From the Mallory Institute of Pathology.

1. Aschoff, L.: Lectures in Pathology, New York, Paul B. Hoeber, Inc., 1924.
2. Gainsboro, H.: Quart. J. Med. **89**:101, 1929.

quently bound in sheaves. They are readily distinguished from cholesterol crystals, since they disappear when the section is heated, to reappear as the slide cools. They arise within fat drops in fat cells or may be found as free extracellular deposits, as in frozen sections of the adrenal gland. Crystalline cholesterol esters are brought out more clearly by moderate heating of the slide.

Under strictly normal conditions massed visible cholesterol in the form of crystalline esters is found only in the cells of the adrenal cortex, the interstitial cells of the testicle and the cells of the corpus luteum. This localization suggests that the crystalline ester is a storage form of the cholesterol available for being converted into the specific hormones. Crystalline ester cholesterol is met with almost constantly within cells. The fluid crystals may be freed when the cells containing them undergo necrosis, but they provoke phagocytosis and are usually rapidly engulfed by macrophages. Or the esters may be split, the fatty acids may disappear and the cholesterol may be precipitated in the familiar rhomboid plates. This reaction serves to identify the esters as cholesterol esters. Technically, phosphatides and cerebrosides may combine with fatty acids to produce spheroidal crystals with Maltese cross markings. Atherosclerotic lesions in man and the rabbit are associated with massed collections of macrophages containing crystalline ester cholesterol. It is possible that individual macrophages met with in the human body may contain spheroidal crystals other than those of cholesterol. However, the occurrence of massed macrophages containing crystals other than cholesterol esters is met with apparently only in the rare Gaucher and Niemann-Pick diseases.

THE KIDNEYS AND CRYSTALLINE ESTER CHOLESTEROL

When rabbits are fed cholesterol for long periods—for example, a year—there may arise cirrhosis of the liver, enlargement of the spleen and chronic nephritis, a triad of lesions looked on by Gye and Purdy [3] as specific for silica poisoning following the intravenous injection of silica sol in rabbits. Gardner and Cummings [4] injected crystalline silica in suspension intravenously in rabbits. Crystals of the order of 12 microns were sifted out of the circulation in the lungs, those of the order of 6 microns were sifted out in the spleen, while those of the order of 1 to 3 microns were removed from the circulation by Kupffer cells in the liver. These macrophages filled with silica crystals tended to plug the periportal lymphatic channels, the contents of the cells stimulated fibrosis and cirrhosis resulted. Similarly crystalline cholesterol esters, esterified

3. Gye, W. E., and Purdy, W. J.: Brit. J. Exper. Path. **3**:75, 86, 1922; **5**:238, 1924.

4. Gardner, L. U., and Cummings, D. E.: Am. J. Path. **9**:751, 1933.

in the rabbit liver, are picked up from the liver cells by Kupffer cells in the form of crystals 1 to 3 microns in diameter. The macrophages, after long feeding of cholesterol, tend to accumulate in and plug the periportal lymphatic vessels. The contained esters stimulate fibrosis, with production of cirrhosis, in the same manner as do silica crystals. There can be no doubt that crystalline ester cholesterol, like silica, is a stimulant to the growth of fibrous tissue. This power is an important factor in the lesions produced in human and experimental rabbit atherosclerosis.

The lesions of chronic nephritis appearing in the rabbit fed cholesterol for long periods follow the accumulation of masses of cholesterophages in the intertubular connective tissue in the upper pyramid near the junction with the cortex. These massed cells suffer from inadequate nutrition and poor physical support. They tend to undergo necrosis, freeing the crystalline esters. The esters are split and solid crystals of cholesterol are precipitated. These stimulate fibrosis, and tubules are caught and strangled in the scar tissue. New deposits of cholesterophages continue to occur and undergo the changes described in such manner that much of the pyramid may come to be occupied by solid cholesterol crystals in scar tissue. The scar tissue strangulation of nephrons results in nephrosclerosis as definite as that produced by primary vascular changes, and can be the cause of death of the animal.

The deposition of cholesterol in the rabbit kidney appears to be selective. The kidney may come to contain more cholesterol than any other organ except the liver. Indeed, when feeding of cholesterol is stopped before cirrhosis is produced, the liver is slowly cleared of the substance, and gradually returns to normal, while cholesterol may increase in macrophages in the renal pyramid during the clearing of the liver.

CHOLESTEROL IN HUMAN RENAL DISEASE

That doubly refracting crystals occur in the human kidney and the urine was first recognized by Kaiserling and Orgler.[5] The crystals were identified as crystalline ester cholesterol by Adami and Aschoff.[6] Windaus [7] found in fresh normal kidney 0.22 mg. of free cholesterol and 0.01 to 0.03 mg. of ester cholesterol per hundred grams. In kidneys showing glomerulonephrititis he found 0.22 to 0.33 mg. of free cholesterol and 0.09 to 0.65 mg. of ester cholesterol per hundred grams.

In lipoid nephrosis associated with hypercholesteremia there are deposits of crystalline ester cholesterol in macrophages in the peri-

5. Kaiserling C., and Orgler, A.: Virchows Arch. f. path. Anat. **157**:296, 1902.
6. Adami, J. G., and Aschoff, L.: Proc. Roy. Soc., London, s. B **78**:359, 1906.
7. Windaus, A.: Ztschr. f. physiol. Chem. **48**:110, 1910.

glomerular and intertubular connective tissue. The quantity deposited may be considerable, as is seen in figure 1 *A*. This shows a frozen section of the kidney of a woman aged 27 years with hypercholesteremia (blood cholesterol rising to 586 mg. per hundred cubic centimeters) who died suddenly during an anaphylactoid seizure following an intravenous injection of mercupurin® (former proprietary name of mercuzanthin, which is now administered as mercurophyllin injection U. S. P.). This section has been stained lightly with methylene blue so that an artery and a vein can be identified on the left and some tubules on the right. The section was warmed and was then compressed between cover glass and slide by means of the rubber on the end of a lead pencil. The macrophages were ruptured, and the cholesterol ester crystals, present in the cells as droplets 1 to 3 microns in diameter, have fused to produce the larger crystals seen.

The commoner human disease in which deposits of crystalline ester cholesterol appear in the kidneys is glomerulonephritis. This disease is usually associated with hypercholesteremia, but the cholesterol is deposited in the tubular epithelium directly, and cholesterophages play a minor part, if any. At first the deposit is in the basal portions of the epithelial cells (fig. 1 *B*), but as glomerulonephritis advances the deposit is diffused throughout the cytoplasm of the cells. In figure 1 *C* there is seen a distal convoluted tubule in a case of advanced glomerulonephritis. The silvery gray appearance of the tubular epithelium is due to the presence of cholesterol ester crystals 1 to 3 microns in diameter. Desquamated cells and fused crystals occupy the lumen. In the early stages the deposition of the crystalline esters may be focal. An accidental finding in early glomerulonephritis of a widney is illustrated in figure 2 *A*. A dilated convoluted tubule shows the lining epithelial cells over two thirds of the circumference swollen and staining deeply with sudan IV. The cells are crowded on the basement membrane and cover a large papillary stem below, and there is a suggestion of papillary beginnings along the right wall. The other third of the lining epithelium is made up of low cells which do not take the fat stain. The suggestion is that something in the sudan IV–staining contents of the cells has stimulated the lining cells to grow. In figure 2 *B*, showing the same section under polarized light, crystalline ester cholesterol is present in large amounts in the cells which take the fat stain and is absent except for accidental deposits in the cells which do not take the fat stain.

The epithelium lining normal renal tubules contains no crystalline ester cholesterol. The ester found in the epithelium of renal tubules in glomerulonephritis almost implies that this disease is associated with or is responsible for a disturbance of cholesterol metabolism. The hypercholesteremia which appears in the disease supports this belief.

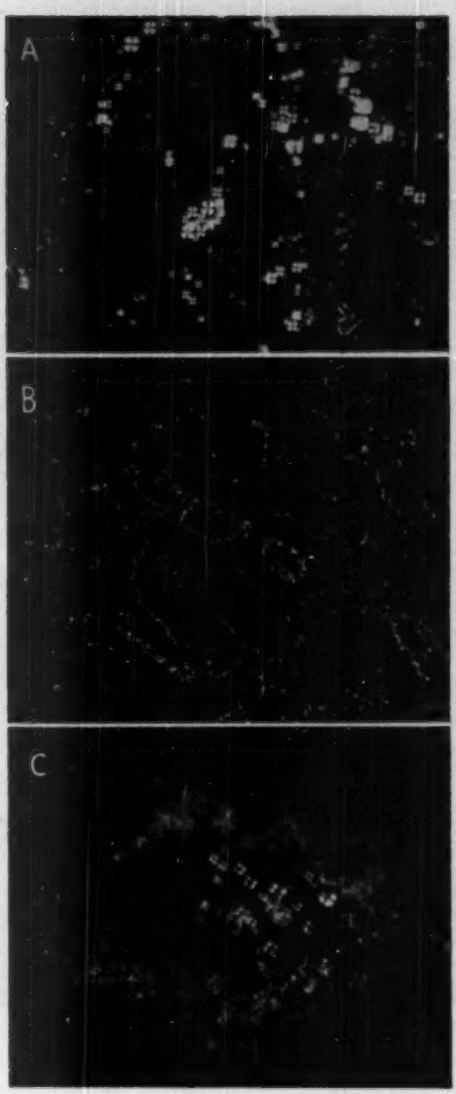

Fig. 1.—*A*, section of a kidney showing lipoid nephrosis, seen under polarized light, after compression. Frozen section; methylene blue stain; × 164.5. *B*, section of a kidney showing glomerulonephritis, seen under polarized light. Frozen section; unstained; × 41. *C*, convoluted tubule of a kidney showing advanced glomerulonephritis, seen under polarized light. Frozen section; unstained; × 164.5.

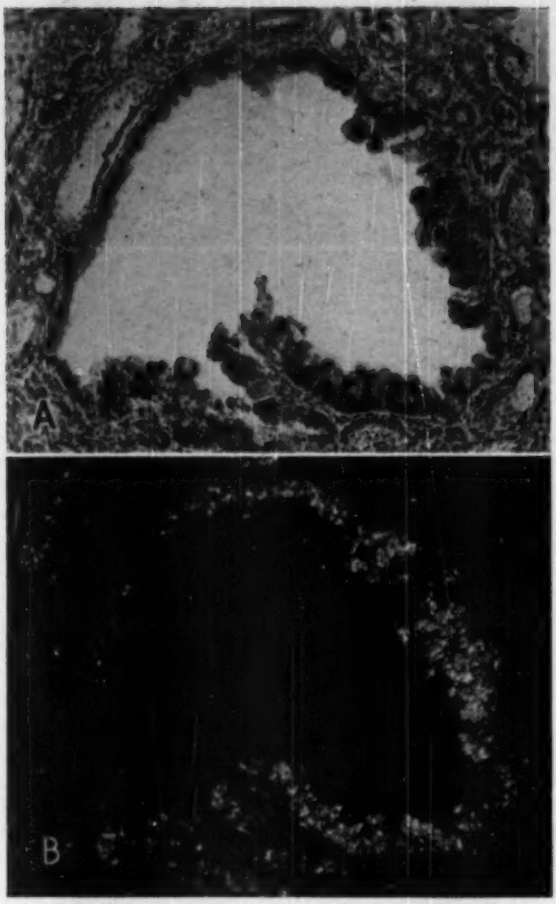

Fig. 2.—Dilated proximal convoluted tubule of a kidney showing early glomerulonephritis: *A*, frozen section; hematoxylin and sudan IV (see text); × 100. *B*, same section under polarized light.

THE RELATION OF CRYSTALLINE ESTER CHOLESTEROL TO THE CORTICAL RENAL TUMORS OCCURRING IN ADULTS

Cortical renal tumors are of two broad types. The so-called Wilms tumor is found in children usually under 7 years of age. The cortex of the kidney may continue to grow after birth in the subcapsular zone by a progression which is evidently a continuation of the embryonic growth of this region, according to Geschickter and Widenhorn.[8] The Wilms tumor is manifestly a tumor (mixed) of embryonic origin and is apparently derived from the primitive metanephric blastema.

The second group—the adult cortical tumors—tend to arise in persons 40 years of age or older. These tumors have always been a problem group. Ackerman and Regato[9] recorded that approximately 99 per cent of the solid tumors are "malignant" and that about 80 per cent of these are adenocarcinomas. A small percentage are alveolar carcinomas. The adenocarcinomas arise in general as papillary cystic tumors or cystadenomas. To quote Geschickter and Widenhorn[8]:

> . . . The malignant cystadenomas have a microscopic structure resembling in general pattern [that of] the benign tubular adenomas . . . . The microscopic distinction between some of these malignant cystadenomas and the benign growths from which they arise may be extremely difficult. There are no outstanding microscopic features by which the two can be distinguished invariably. It is a far safer rule to regard all cystadenomas over 4 cm. in diameter as malignant. While the growth of these neoplasms is slow, extensive metastases are the rule. Thrombi of cells may be found in the veins, metastases to the lungs and abdominal viscera occur, and the bones may be involved by secondary deposits.

It is agreed that the only apparent difference between the more complex adenomas and the adenocarcinomas may be that the cancerous forms metastasize, and the adenomas do not. Bell[10] concluded that the danger of metastasis began with tumors 5 cm. in diameter; later he modified this to 4 cm. in diameter.[11]

The kidney is remarkable in that miniature tumors, usually millimeters in diameter, may be found in relation to its cortex. Three epithelial types are found. Adrenal rests occur, lying on the capsule or in it, or rarely under it. They are usually small, tend to be flattened, are orange in color, are made up of cords of cells rich in cholesterol esters and on the whole rarely develop into frank adrenal tumors (carcinomas). A second but rare nodule—opaque gray—is made up of large polygonal cells, with large nuclei, occurring in solid alveolar arrangement. Such a nodule appears to be an early stage of an alveolar carcinoma. The

8. Geschickter, C. F., and Widenhorn, H.: Am. J. Cancer **22**:620, 1934.

9. Ackerman, L. V., and Regato, J. A.: St. Louis, C. V. Mosby Company, 1947, p. 696.

10. Bell, E. T.: J. Urol. **39**:238, 1939.

11. Bell, E. T.: A Text Book of Pathology. Philadelphia, Lea & Febiger, 1944.

commonest miniature new growth is the papillary adenomatous or cystadenomatous nodule which arises from renal epithelium as I shall demonstrate. This type of miniature growth may occur singly or in small numbers or in great numbers in the kidneys of persons, largely men, who are 30 years of age or older (usually 40 years plus) and whose kidneys commonly present evidence of nephrosclerosis.

MATERIAL OF PRESENT STUDY

Miniature tumors have been collected at the Mallory Institute of Pathology over a period of years. Solitary growths, nodules appearing in small numbers and particularly kidneys showing large numbers of nodules make up the material which has been studied. The richest supply has come from the kidneys containing multiple

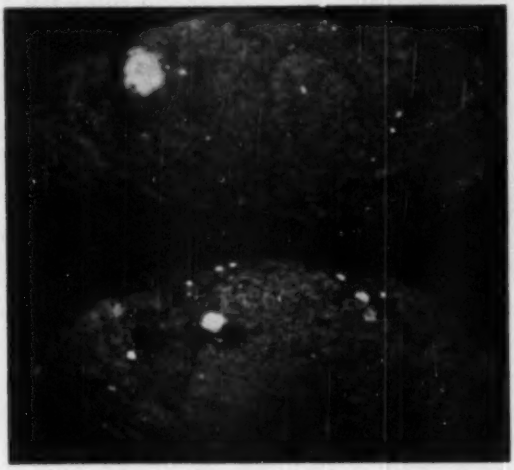

Fig. 3.—Nephrosclerotic kidney showing multiple miniature tumors.

nodules (fig. 3). A total of more than 300 miniature tumors from kidneys showing solitary nodules or multiple nodules furnishes the basis of this portion of the study.

THE SEQUENCES OF MINIATURE TUMOR GROWTH

Particularly in kidneys showing multiple small tumors, microscopic studies brought out the presence of tumors too small to be seen with the naked eye. There began to emerge from the series of sections the evidence that we were viewing a quite constant sequence of stages in the evolution of these tumors. Furthermore, there appeared evidence of a forerunner of actual tumor formation, which marked off portions of a proximal convoluted tubule from the rest of the tubule and from neighboring tubules.

This appearance is illustrated in figure 4. It begins with the focal deposition in the epithelial cells of a tubule of a material staining as fat (fig. 4 *A*), which may be limited to a single fold of the tubule as in this case. Under polarized light (fig. 4 *B*) the affected cells contain a finely granular, doubly refractive material. If the slide is warmed and pressure brought to bear on the cover glass the effect seen in figure 4 *C* will appear. The minute granules from figure 4 *B* have fused into larger drops and show the typical markings of crystalline ester cholesterol.

Deposits of crystalline ester cholesterol may be limited to a single fold of a tubule or may include several folds. Apparently, the deposit leads to stimulation of connective tissue, which grows in above and below the portion or portions of the tubule the cells of which contain the ester crystals. This serves to segregate the affected portions of the tubules. If the crystalline deposit is limited to one fold of the tubule, that fold will be isolated. If the lining cells of many folds contain ester crystals, each of the folds will be segregated from the others and from the rest of the tubule.

The result of the segregation is the sealing of the affected segments into closed sacs. There is no provision for drainage. The secretions of the lining cells may appear in bubble-like globules in the free ends of some cells in some segments. At any rate the secretion accumulates and dilates the lumen to form a cyst. Figure 5 *A* illustrates a small early cyst lined by cells of varying size, the contents of which stain deeply with sudan IV. In a lesion made up of a larger and some smaller cysts, the doubly refractive contents of the cells are brought out under polarized light, and cholesterol ester crystals have been dislocated in making the frozen section. (fig. 5 *B*).

As the cysts enlarge, the new growth of lining epithelial cells keeps pace with the increase in size, as does the supply of crystalline ester cholesterol. Indeed, the stimulus to the growth of the lining epithelium is such that the production of new cells tends to outrun the increase in the size of the cyst. This causes crowding of the lining wall to the degree that provision of new space for the cells becomes necessary. This is accomplished by the formation of papillae projecting into the lumen. In figure 5 *C* are seen two blunt papillary projections accommodating the new cells. One has here the first steps in the production of a papillary tumor.

In figure 6 *A* there appear multiple isolated segments of a proximal convoluted tubule in which the deposit of ester crystals occurred throughout the multiple folds of the tubule. There are evident multiple layers of lining cells with cytoplasm taking the fat stain, contrasting with the cells lining neighboring normal tubules. Irregular papilla formation has already begun. In figure 6 *B* larger multiple cysts of similar origin are seen, some with early papilla formation. The lesions surround a large subcapsular vein.

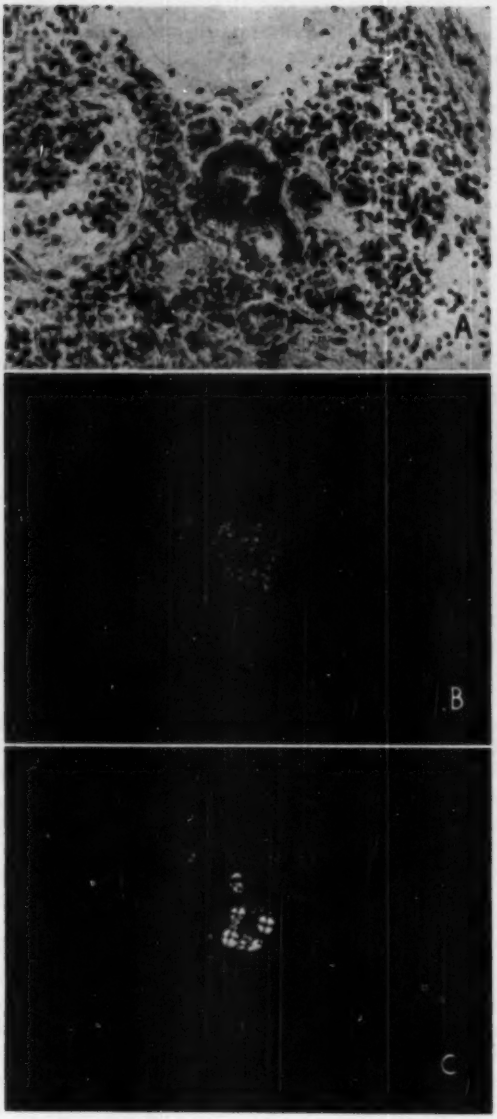

Fig. 4.—Proximal convoluted tubule of a nephrosclerotic kidney with fatty contents in epithelial cells: *A*, frozen section; hematoxylin and sudan IV; × 100. *B*, same field under polarized light. *C*, same field after the slide had been warmed and compressed to free cholesterol ester crystals.

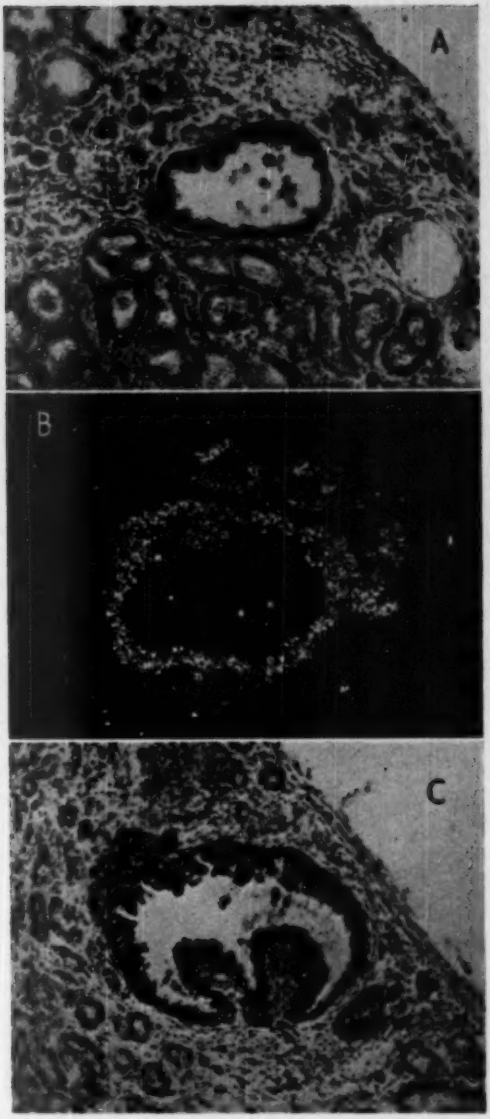

Fig. 5.—*A*, dilated single segment of a proximal convoluted tubule lined by cells with sudan IV–staining contents. Frozen section; hematoxylin and sudan IV; × 90. *B*, cystic convoluted tubule in one large and three smaller segments. Note crystalline cholesterol crystals. Frozen section; unstained; under polarized light; × 70. *C*, early papillary ingrowths in a cyst with crowded cells along the walls. Frozen section; hematoxylin and sudan IV; × 80.

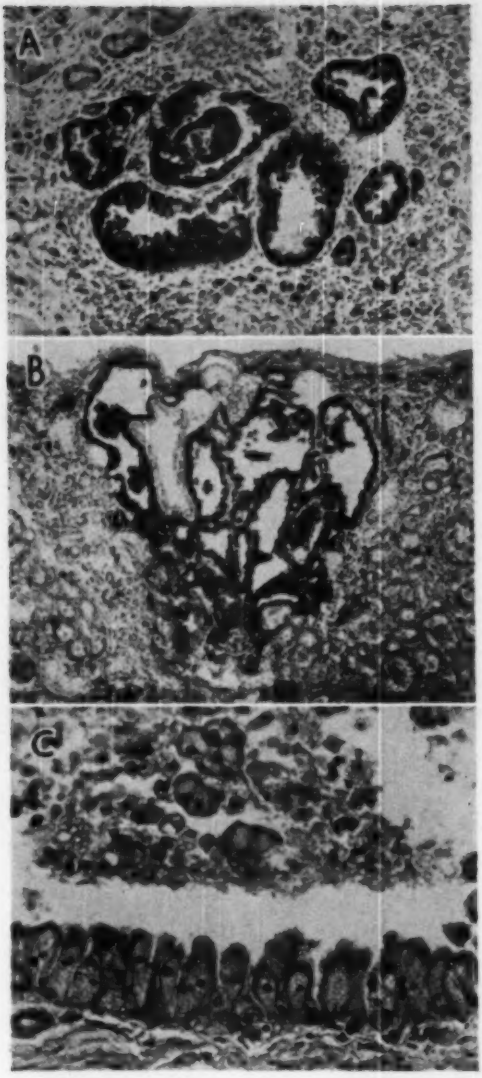

Fig. 6.—*A*, multiple folds of a proximal convoluted tubule with cells containing sudan IV–staining material that have been segregated (see text). Hematoxylin and sudan IV; × 75. *B*, multiple cystic spaces lined by cells rich in contents taking the fat stain. Frozen section; hematoxylin and sudan IV; × 40. *C*, cells lining a cystic convoluted tubule; the fats have been extracted, leaving foam cells. Tissue fixed in Zenker's fluid and embedded in paraffin; Mallory's phosphotungstic acid–hematoxylin; × 400.

Figure 6*C* reproduces part of the wall of a large cyst, paraffin embedded. The fine vacuoles in the foamy cytoplasm contained cholesterol ester crystals of about the same size as those occurring in foam cell macrophages. Desquamated cells show similar foamy cytoplasm. The general resemblance which the epithelial new growth bears to embryonic tissue is brought out in figure 7*A*, in a paraffin-embedded section of an early papilla. The ends of some cells show granules of hyaline degeneration.

The cortical position of these developing tumors is illustrated in figure 7*B*. The projecting papillary stems are largely simple, but branching is beginning in places. The complexity of the branching processes, as the tumor grows, is made plain by the skeleton-like appearance, with branching stems and secondary cysts, shown in an unstained section of a larger tumor (fig. 7*C*). The crystalline esters are limited to the epithelial layer except for the crystals seen in the macrophages in the vein to the right and in another below. Ultimately the papillary processes fill the cystic space and produce what is grossly a solid tumor (fig. 8*A*). Three minute solid papillary tumors have been formed and are seen below the main tumor.

Figure 8*B*, representing a grossly solid tumor, illustrates that together with the epithelial deposition of esters there is a constant macrophagic removal of excess esters. In this photomicrograph taken under polarized light—with sufficient diffuse lighting to bring out the pattern of the tumor—there are evident crystals in the epithelial cells, partly obscured by the introduction of diffuse light. There are in addition concentrated masses of crystals in foci, which are lymphatic channels filled with macrophages.

A continual flux of ester cholesterol crystals, into new cells of the tubular epithelium and out of degenerating cells into macrophages, appears to be the rule. As far as observation goes, the deposition of crystals, at least in the early stages, is direct, apparently from blood sources. There is no evidence of other cells in the early deposits. One thinks of esters being precipitated within the epithelial cells from invisible forerunners, as probably occurs in the case of the deposits in cells of the adrenal glands, the testicle and the corpus luteum. The removing of the crystals from tumors, on the other hand, requires the activity of scavenger cells, the macrophages. The stability of the ester crystals is worthy of note. The crystals tend to persist in macrophages until the cells die. The selection of this form of cholesterol to serve as a reserve supply of material in the tissues that produce the steroid hormones of the adrenal cortex and the gonads is probably due to this stability. Apart from the tissues in which the crystalline esters are normally stored they become an irritant substance and are treated as foreign bodies.

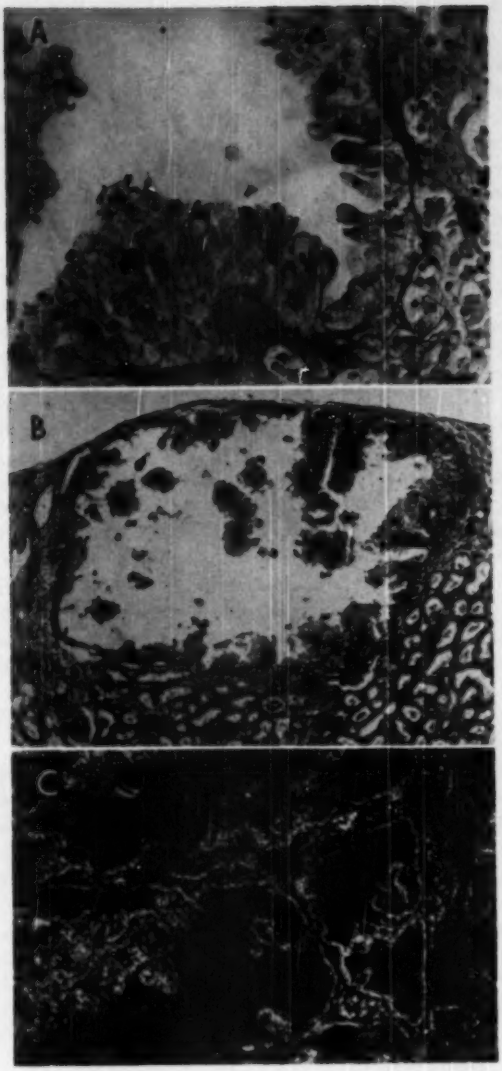

Fig. 7.—*A*, early papillary formation in a cystadenoma with an embryonic type of cell growth. Tissue fixed in Zenker's fluid and embedded in paraffin; Mallory's phosphotungstic acid–hematoxylin stain; × 342. *B*, cystadenoma with multiple papillary stems. Tissue fixed in Zenker's fluid and embedded in paraffin; Mallory's phosphotungstic acid stain; × 57.5. *C*, larger, almost solid tumor, under polarized light. Crystals, limited to the epithelium, illuminate the growth pattern. Frozen section; unstained; × 36.5.

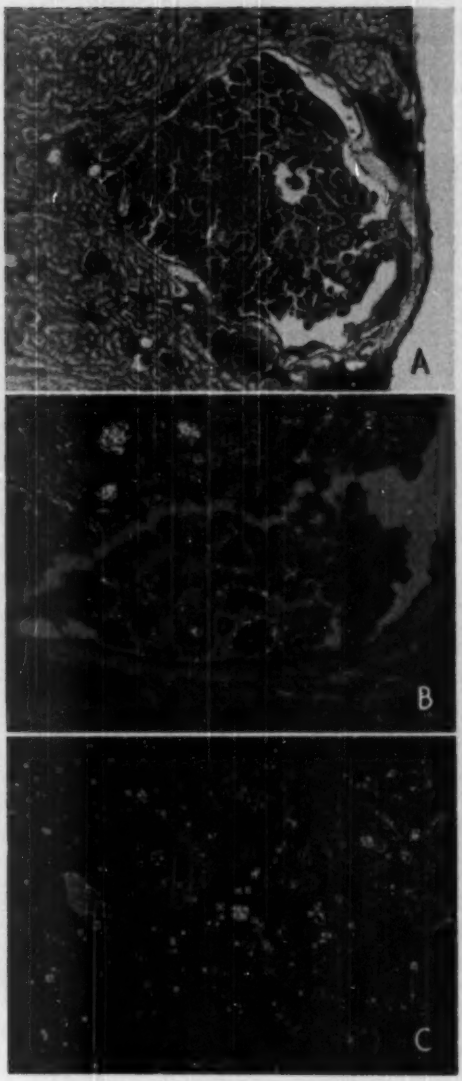

Fig. 8.—*A*, grossly solid papillary tumor with three small satellite tumors. Frozen section; hematoxylin and sudan IV; × 28. *B*, solid tumor under polarized and diffuse light. Note distended lymphatic channels to the left and above. Frozen section; hematoxylin and sudan IV; × 56.5. *C*, larger tumor under polarized light. Frozen section; unstained; × 80.7.

To return to the growing tumor, papillary growth leads to the filling of the cystic space or spaces. As the tumors progress and become more complex, the papillary pattern of the growth may become less obvious. There may arise glandlike acini or tubules which suggest more

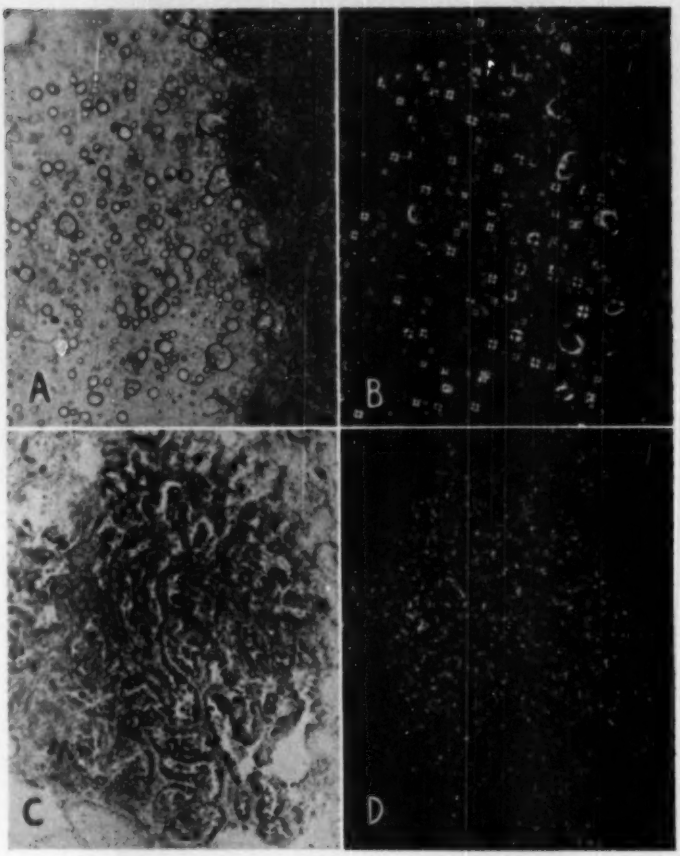

Fig. 9.—Crystalline ester cholesterol expressed from miniature tumor: *A*, greaselike drops. Unstained section; diffuse lighting; × 100. *B*, same field under polarized light. *C*, frozen section of miniature tumor, showing pattern of growth. Hematoxylin and sudan IV; × 70. *D*, unstained frozen neighboring section of same tumor under polarized light.

definitely the type of cell grouping seen commonly in cancerous cortical tumors. Figure 8*C* shows an unstained section of a larger miniature

tumor, in which the pattern of glandlike growth is brought out under polarized light, as is the character of the crystals.

The crystalline content of the growing tumor and the character of the crystals can be demonstrated by forcing the crystals out of the tissues. In figure 9 *A* is seen the edge of an unstained section of a miniature tumor from which the esters were expressed by rolling a glass rod over the tissue. The esters are seen as soft greaselike drops of varying size. In figure 9 *B* the typical markings of the crystal images are manifest. The large masses at the tumor edge are compressed between cover glass and slide, have lost symmetry and show atypical images. Also evident is the purity of the crystalline deposit, as is constant in the miniature tumors.

DEVOLUTION INTO TUBULAR ADENOMAS

Thus far the tumors dealt with in this article have been those showing progressive growth. A large percentage of miniature tumors progress to a certain point and then regress into inactivity. Characteristic of the progressing tumors is their epithelial cell content of sudan IV–staining material and crystalline ester crystals. These establish the pattern of the growth. Figure 9 *C* from a stained frozen section illustrates the pattern of a papillary new growth of standard type. Figure 9 *D*, from an unstained neighboring section under polarized light, reproduces in general the pattern of figure 9 *C* with some changes, because of the difference in the sections. In figure 10 *A* a tumor nodule, less advanced, is made up of early papillary stems. Under polarized light (fig. 10 *B*) the deposit of crystalline esters does not follow the pattern of the tumor. The appearance suggests the casting out of the crystalline esters, which tend to be concentrated in the lower midregion of the tumor, perhaps on their way to removal.

The common method of removing the esters is more deliberate. Macrophages take over the esters from the epithelial cells and carry them into the lymphatic channels. This is illustrated on a grand scale in figure 10 *C*, in which the lymphatic channels are distended with macrophages. The portions of the tumor that are visible are delicate papillary structures covered by thin, flattened cells without fat-staining contents, and showed no anisotropic contents under polarized light.

The end result associated with the removal of the esters is the cessation of growth other than the fusing of the papillary stems with neighboring walls to produce a glandlike structure. An early stage of this metamorphosis is illustrated in figure 11 *A*. There still remain a few papillary stems, but the frozen section disclosed little material taking the fat stain, and no ester crystals appeared under polarized light.

Figure 11 *B* illustrates the dead end character of a tubular adenoma which has lain dormant in the kidney long enough to become encapsulated

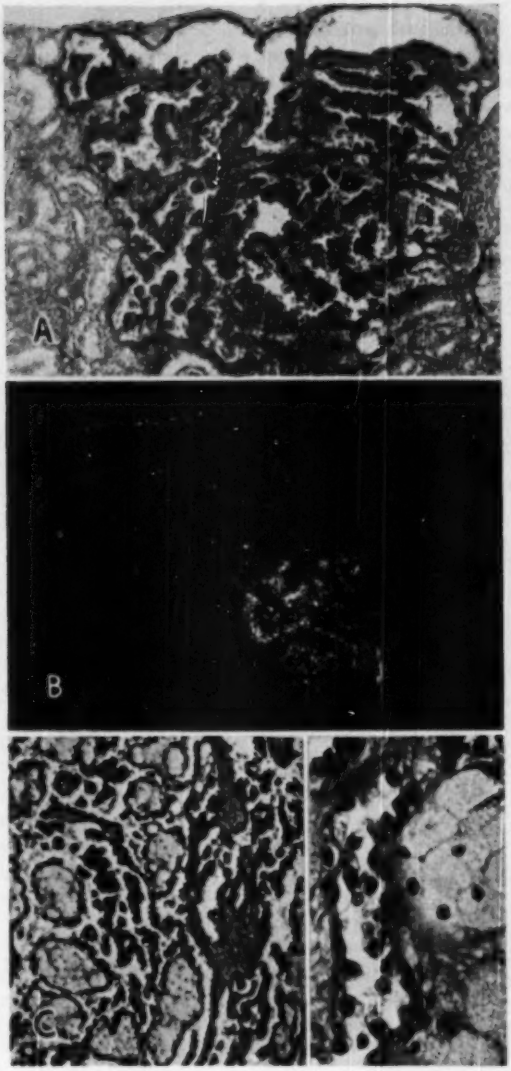

Fig. 10.—*A*, early papillary new growth. Frozen section; hematoxylin and sudan IV; × 100. *B*, same field under polarized light. *C*, shows crystalline ester cholesterol being removed by the macrophages (foam cells) distending the lymphatic channels in a miniature tumor. Tissue fixed in Zenker's fluid and embedded in paraffin; Mallory's phosphotungstic acid-hematoxylin; × 70. At right, enlargement of macrophages in lymphatic channels; × 400.

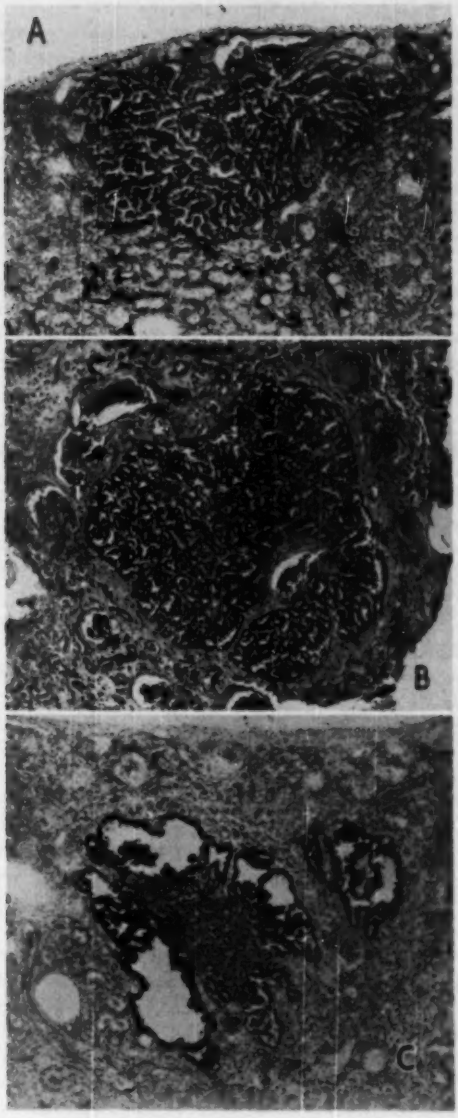

Fig. 11.—*A*, early tubular adenoma. Frozen section; hematoxylin and sudan IV; × 60. *B*, encapsulated tubular adenoma. Tissue fixed in Zenker's fluid and embedded in paraffin; hematoxylin and eosin; × 70. *C*, fresh deposits of sudan IV–staining material about an inactive tubular adenoma with production of cysts. Frozen section; hematoxylin and sudan IV; × 40.

by hyaline connective tissue. Its cells are shrunken so that they are mostly nuclei, as Ewing has said, and show no sudan IV–staining material or crystalline esters. It is probable that some of the so-called fibromas of the renal cortex arise from such tubular adenomas whose capsules of dense connective tissue have contracted and cut down the circulation, with resulting death of the epithelial cells. Only the supporting tissue survives.

At a glance the lesion seen in figure 11 *C* appears to be a reactivation of a tubular adenoma. Careful scrutiny, however, makes plain that the cells of the original tubular adenoma, in contrast to the series of surrounding active cystic processes, did not take the fat stain, nor did they contain anisotropic material under polarized light. There is a distinct suggestion that the original tissues of the tubular adenoma have shown resistance to the redeposit of crystalline esters of cholesterol.

ADULT CORTICAL RENAL ADENOCARCINOMA

The tumors of the renal cortex occurring in adults are remarkable in that pathologic formations varying in appearance from that of a relatively simple cystadenoma to that of a complex carcinoma may be found in the same tumor. Papillary structures of various types, adenoma formations varying from cystic glands to finer glands, coarse papillary masses without adequate supporting tissue, adenomatous structures with multiple layers of cells and fields in which such masses are converted into solid alveoli may be present. Hemorrhage with necrosis and necrosis without hemorrhage are the inevitable result of growth so rapid that adequate blood supply and adequate physical support are not provided.

Most of the lesions evidently arise from abnormal changes in epithelial tissue, but changes in the supporting tissues may appear when the epithelial type cells swell, become clear, die and disappear. The delicate strands of intercellular tissue may survive and produce a picture suggesting the primitive metanephric blastema. Or there may be formed a more compact sarcomatoid tissue, in which necrosis is prone to occur except immediately about the larger vessels, due apparently to an inadequate capillary system. Or the supporting tissue may mature with the formation of frank adult fibrous tissue.

The color in the cut surface of the tumor may vary from orange where the tissue is richest in crystalline content through yellow where crystalline material is less abundant, to white, particularly where sarcomatoid changes, poor in crystalline material, have arisen. As the tumor ages, tissues may become free of cholesterol crystals, particularly in older portions.

Some observers classify renal cortical tumors of adults into three groups—granular cell tumors, mixed tumors and clear cell tumors.

This classification is based on the study of slides from embedded tissue. The fat solvents used in the process of embedding dissolve lipids, including crystalline ester cholesterol, leaving behind the skeletal portions of the cell cytoplasm which give the cells a granular appearance. The possible source of the clear cells may be the solution of lipids in sudan IV–staining material as is illustrated in figure 12 *A* and *B* from a frozen section of a portion of a renal adenocarcinoma that showed cystic glands. In figure 12 *A*—stained for fat—the cells lining the glands are high and stain deeply with sudan IV. The same field under polarized light (fig. 12 *B*) shows the apparent incomplete removal of the doubly refringent material of the cells, leaving behind some atypical crystals. The appearance suggests the possibility that some crystals had undergone rapid solution and that others, more resistant, persisted. The process of solution was caught in the midst of the solvent action.

In the prevention of atherosclerosis of the arteries of youth, the cholesterol ester crystals already deposited in macrophages in the intima (in infancy, puberty, etc.) are taken over from the cholesterophages by fibroblasts, and the crystals are dissolved in a material which takes the fat stain and which in the early stages occurs in the bodies of the fibroblasts in irregular-sized but generally large drops. Later in the process the fat-staining material is apparently distributed through the bodies of the fibroblasts as though homogenized in the cytoplasm. This is followed by apparent cellular metabolism that causes the fat-staining material to disappear. There is no evidence that the material is removed by way of the blood or lymph outlets. When the process is completed, the bodies of the fibroblasts, swollen with the fat-staining contents during the process, return to their slender bipolar shape and size.[12]

Another interesting change has been observed in the doubly refringent material found in cells of some cancerous cortical renal tumors. For the most part, doubly refringent material is present in the form of typical cholesterol crystals. There may be bacillary or other forms of crystals in some tumors, but heating the slide usually results in their conversion into typical cholesterol ester forms. However, sometimes variants occur which do not react to the application of heat and remain atypical. Such doubly refractive crystals are seen in figure 12 *C* from a cortical adenocarcinoma. Every cell of the new growth in this field contained crystals. The spaces that are seen in this section were regions of hemorrhage. The pattern of the tumor growth was reproduced by the deposited crystals. Under higher power (fig. 12 *D*) no typical ester crystals are present. The crystals conform to an apparently pure amorphous type.

12. Leary, T.: Arch. Path. **37**:16, 1944.

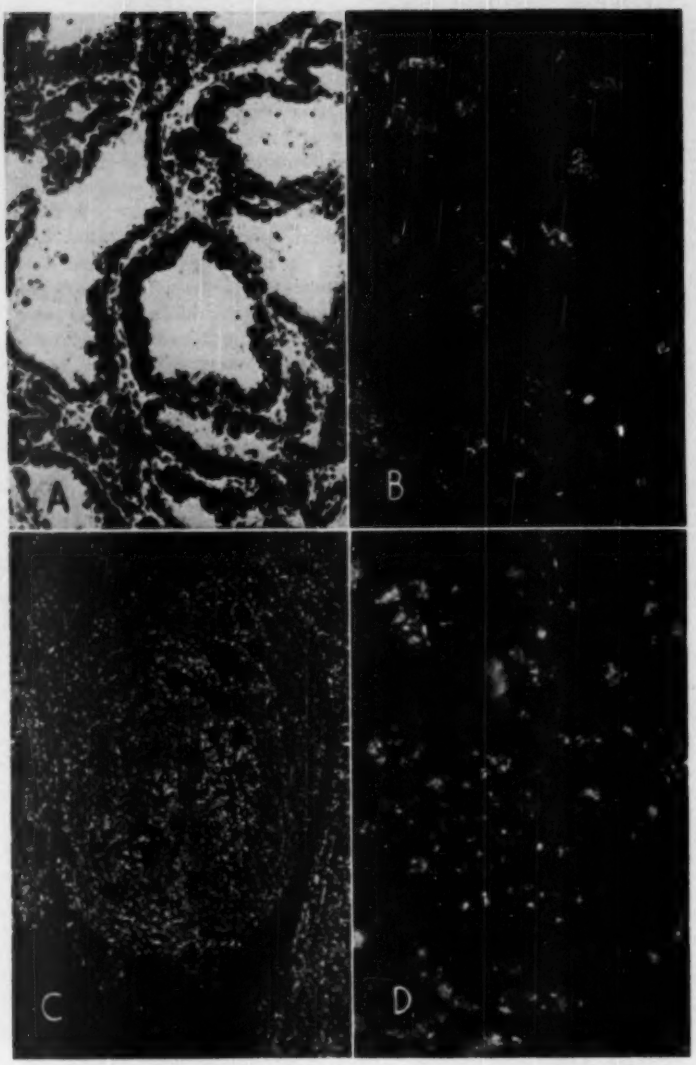

Fig. 12.—*A*, coarse adenomatous structure occurring in a renal cortical adenocarcinoma. Frozen section; hematoxylin and sudan IV; × 100. *B*, same field under polarized light. *C*, pattern of renal cortical adenocarcinoma reproduced by crystals under polarized light; × 70. *D*, enlargement of crystals from same field; × 300.

COMMENT

Production of Benign Adenomas.—Geschickter and Widenhorn [8] concluded that the cortical origin of the adult adenocarcinomas supported a belief that the growths arose from isolated foci of embryonic tubules persisting in the cortical zone. Their illustration of a coiled tubule with deeply staining cell cytoplasm, "recalling the structures in the normal embryonic kidney," corresponds in appearance to the structures described in the present studies (see fig. 6*A*). The so-called embryonic rests of atypical tubules are shown as if they were independent entities and not portions of continuous tubules. In the present studies of the earliest lesions of affected convoluted tubules the neighboring portions of these tubules free from sudan IV–staining material and crystalline ester deposit revealed no abnormality.

A more reasonable source of the benign adenomas is the series of changes reported by Oliver [13] as occurring in chronic hemorrhagic glomerulonephritis. By maceration of formaldehyde-fixed kidneys he found that snipping off of glomeruli from nephrons occurred by the growth of connective tissue. This change also occurs in nephrosclerosis. The separated tubular portions of nephrons either atrophy or persist with hypertrophy if a circulation by-passing the glomeruli is established. The tubules may survive *in toto* or in fragments and may show hypertrophy. That such hypertrophied tubular fragments might serve as depositories of crystalline esters of cholesterol is a not unreasonable assumption.

The present studies differ from those published in that they reproduce a series of apparently sequential lesions such as might occur if nature were conducting a human tumor research and one were privileged to sit in and observe the results.

The sequence leading from the deposit of cholesterol ester crystals in renal epithelial cells to the tubular adenomas seems to be orderly, as presented, and is based on abundant material. The illustrations reproduce a representative sample of the material studied, and were selected merely to make clear the constancy of the stages in the evolution and devolution that marked this portion of the subject. It is possible that some substance other than crystalline ester cholesterol was responsible for the progression and accounted, by its absence, for the lesion's retrogressing into the backwaters of tubular adenomas. However, the activities of crystalline ester cholesterol as a stimulant of connective tissue growth have been discussed, and the possibility, and even probability, that this substance could stimulate epithelial growth if properly placed is a reasonable deduction from the evidence.

13. Oliver, J.: Arch. Path. **18**:777, 1934.

Some factor in cholesterol metabolism leads to the deposit of visible crystalline esters in the kidney, particularly in renal epithelial cells, and even more particularly in cells of the convoluted tubules when the kidneys are diseased. The facts that the esters are present in the renal epithelium before new growth appears, that the early new growths are limited to the focal tissues in which the esters have been deposited, that there is a progressive increase in the quantity of cholesterol esters limited to the tissues of the new growth as the tumor waxes in size, that the removing of the crystalline esters from the growing tumor is followed by stoppage of growth and that the formerly active tumor literally declines into innocuous desuetude all point directly to crystalline ester cholesterol as a probable dominant factor in this sequence.

Adult Renal Cortical Adenocarcinoma.—There is apparent unanimity in the conclusions of students of the subject that this, the common type of cancerous renal cortical tumor in adults, is derived from early benign adenoma of the organ. To this point I have followed the progress and the stoppage of progress in the formation of tubular adenomas. Certain of the benign adenomas do not regress. If they reach the size of 4 cm., they may be converted into adenocarcinomas, which may retain in places the evidence that they have progressed from papillary cystadenomas.

The discovery that tumors contain crystals of cholesterol esters is not new. Chemical reports of increased amounts of cholesterol in tumors have been many, particularly in the decade from 1930 to 1940. The outstanding physicochemical study of its presence is that of White.[14] He used a warm stage by which controlled temperatures could be obtained from 15 to 95 C. Under polariscopic lighting he observed five types of crystals, the melting point and the physical changes of which under heat could be recorded. Of the five types, type A was proved to be made up of solid crystals of cholesterol in the characteristic rhomboid plates. Spheroidal fluid crystals with the Maltese cross markings and rod-shaped forms that changed into typical spheroidal crystals under heating made up crystals of types B, D and E and were combinations of cholesterol with lecithin, fatty acids or other substances. Type C crystals were of ordinary neutral fats that melted between 45 and 55 C. and solidified sharply at temperatures identical with or a little below the melting point.

White found cholesterol crystals in most carcinomas examined, though in some cases they were present only in metastatic nodules. These tumors included growths from lip, tongue, pharynx, breast,

14. White, C. P.: J. Path. & Bact. **13**:3, 1908.

stomach, pancreas and liver. Crystals were also present in sarcomas, a chloroma, and in adenomas. He concluded: "It is suggested that cholesterin may be associated in some way or other with the regulation of cell proliferation."

Bierich and Lang [15] reported that the determination of the cholesterol contents of tumors removed surgically was of definite prognostic value. From a follow-up of 64 tumors the following data were obtained. When the cholesterol content of the tumor tissue was 150 mg. per hundred grams or less, all patients were living at the end of four years. When the quantity was 150 to 250 mg. per hundred grams, 47 per cent of the patients survived for four years. When the amount present was 250 to 350 mg. per hundred grams, 12 per cent survived for four years. When the content was 350 mg. per hundred grams or more, no patients were living at the end of three years, and 80 per cent died within one year.

Carcinogens.—Why should a benign papillary adenoma be converted into an adenocarcinoma when the adenoma grows to a size of 4 cm. or over? The obvious answer to this question would be the presence or the development of a carcinogen.

Present knowledge of chemical carcinogens began with the work of E. L. Kennaway and his associates. Yamagiwa and Ichikawa [16] had demonstrated that coal tar painted on the skin of the rabbit's ear would produce papillary growths and cancer. The Kennaway group tried to find the chemical agent responsible for this cancer causation. Incidental to this study, but not a part of it, was the discovery that 1,2,5,6-dibenzanthracene would produce cancer in mice. Later it was ascertained that dibenzpyrene was apparently the carcinogenic agent in coal tar. This work opened up a new field of chemical and experimental study of carcinogens and their mode of action.

The fascinating possibilities of clearing up the causation of man's perhaps most mysterious disease led to mass experimental research. Literally hundreds of thousands of mice were used for experimental purposes, in addition to rats, rabbits and other laboratory animals. Out of this work came evidence of the carcinogenic potency of many chemical substances. The early work was associated particularly with agents responsible for industrial hazards, such as coal tar and azo and aniline dyes, whose causal agents were known. The carcinogens isolated in the early work were potent and produced tumors at the site of inoculation or in remote organs in a high percentage of inoculated mice.

15. Bierich, R., and Lang, A.: Klin. Wchnschr. **151**:667, 1936.
16. Yamagiwa, K., and Ichikawa, K.: J. Cancer Research **3**:1, 1918.

Voegtlin,[17] as a result of wide experience in the experimental field, offered the following tentative picture of the mode of action of carcinogens:

> . . . Chemical carcinogenic agents acting in optimum concentration upon genetically susceptible cells set in motion continued cellular proliferation. This first phase does not necessarily give rise to cancer cells and under certain conditions may be reversible. Because of their high degree of lipoid solubility the carcinogens pass into the cell membrane. The unstable equilibrium of cells undergoing division presumably favors gradual alteration during repeated cycles of cell division until the malignant change is completed.

Conclusions drawn from the study of carcinogens have been based for the most part on the results of animal experiments with the more powerful carcinogens. The average human cancer has little to compare with the near constancy with which mouse cancer follows the injection of certain carcinogens and the rapidity of growth which the tumor displays once it is established. The dramatic character of the findings in mice following the injection of benzpyrene, for example, is not duplicated in the average human cancer. Doubts have been expressed whether one is justified in transferring to human problems, without supporting evidence, the effects of animal experimentation, notably with reference to the human cancer problem.[18]

The known and suspected causes of the cancers observed in experimental animals can be roughly divided into the following groups, according to Willis[19]: (1) The chemical carcinogens, particularly the industrial group; (2) radiant energy, including roentgen radiation, radium, solar and ultraviolet radiation; (3) parasites, including Spiroptera neoplastica (Fibiger-rat) and Cysticercus fasciolaris (rat liver); (4) viruses, namely, the Rous chicken sarcoma virus, Shopes's rabbit virus and Lucké's frog virus; (5) foreign bodies, notably dusts from tarred roads, silica, iron oxide and aluminum oxide, arsenic, etc.; (6) sex hormones.

Human cancer usually comes on slowly, insidiously, and the principal medical problem is that of recognizing the disease in its early stages, when it is localized and amenable to treatment. Physicians can exclude in most cases industrial hazards, radiation, parasites, known viruses and dusts except possibly in cases of lung carcinoma.[20] The distinct suggestion is that something already present in the body is an

17. Voegtlin, C.: Proc. Inst. Med. Chicago **14**:454, 1943.

18. Fieser, L. F.: Hydrocarbon Carcinogenesis Research Conference on Cancer (1944), Washington, D. C., American Association for Advancement of Science, 1945, p. 116.

19. Willis, R. A.: Pathology of Tumors, St. Louis, C. V. Mosby Company, 1948.

20. Campbell, J. A.: Brit. M. J. **2**:179, 1943.

important factor in the causation of most human cancers. Something the metabolism of which becomes less efficient with age would best account for the gradual onset of the disease.

The possibility that cholesterol may be a carcinogen has been brought forward by Hieger in the recent period as the result of experimental work on mice.[21] His studies disclosed that in extracts of human and animal organs "the carcinogen is found in the unsaponifiable fraction, which contains c. 85 per cent of the digitonin precipitable sterol. . . . Commercial cholesterol containing 95-98 per cent of the pure sterol is carcinogenic, and the potency is of the same order as that of the active fractions from human tissue." He reported on a total of 69 sarcomas that developed at sites of injection of lipid substances in approximately 2,000 mice, and on 25 sarcomas in 436 mice, following injections of commercial cholesterol. The average latent period was about eighteen months. He commented:

> . . . The experiments described here suggest the question, Why has it required 20 years, that is, since the discovery of the carcinogenic hydrocarbons in 1929, to show that commercial cholesterol is carcinogenic? The answer is obvious: Investigators in chemical carcinogenesis have become accustomed to the use of powerful quick-acting carcinogens, and as a result tumor inductions by slow-acting carcinogenic substances have been dismissed as non-specific.

In his summary he states that

> . . . the widespread occurrence of a carcinogenic factor in unsaponifiable fractions from many biological sources suggests that it is either cholesterol itself, or a combination of cholesterol and a small proportion of a frequently occurring co-carcinogen.

Because of the minute amounts of certain powerful carcinogens needed to induce sarcomas, it is necessary to be cautious in drawing conclusions. Hieger called attention to the possibility that the laboratory may become contaminated with benzpyrene from air-borne soot. He is trying to produce pure cholesterol in order to determine whether it is the exclusive active agent in the process.

However, the association of cholesterol and the sarcomas induced with human tissue extracts is apparently constant in Hieger's studies.

There is also another possibility. Cholesterol, the mother substance of the adrenal cortical hormones and of sex hormones, together with the bile acids, has many potencies. Methylcholanthrene has been produced in the laboratory from desoxycholic acid and cholesterol and is one of the most powerful of the quick-acting carcinogens. The degree of malignancy manifested in some human renal adenocarcinomas may doubt that the tumor had its origin from a papillary cystadenoma. The be very high in places, together with contrasting fields that leave no

21. Hieger, I.: Brit. J. Cancer **3**:123, 1949.

possibility that a simple papillary adenoma changes to a cancerous new growth when a miniature cortical renal tumor reaches a diameter of 4 cm. suggests the influence of a more active carcinogen than unchanged cholesterol. The cholesterol present in the lesion may be capable of being converted under abnormal stimuli into sex hormones or into methylcholanthrene, for example, which are recognized carcinogens.

SUMMARY

Evidence is presented that the crystalline ester cholesterol deposited focally in the epithelium of renal convoluted tubules in adults, particularly in those afflicted with nephrosclerosis, is the stimulating agent responsible for the growth of benign cortical adenomas in man. Removal of the crystalline ester cholesterol is followed by a cessation of the growth of such adenomas, which then revert to inactive tubular adenomas.

It is generally accepted that adult renal cortical adenocarcinomas have their origin in benign adenomas.

Recent experimental chemical studies (Hieger) are discussed that support the thesis that cholesterol by itself or in combination with small amounts of a co-carcinogen is the cause of the sarcoma induced in mice by the injection of cholesterol-rich extracts of human or animal tissue.

The combined histochemical and chemical evidence leaves little doubt that cholesterol is intimately related to certain human and experimental tumors.

EVALUATION OF CHOLINE IN THE PREVENTION OF EXPERIMENTAL ATHEROSCLEROSIS

Importance of Changes in Body Weight

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SEVERAL investigators have been impressed with the action of choline in retarding or preventing the development of atherosclerosis produced by cholesterol feeding. Steiner [1a] demonstrated that choline could prevent atherosclerosis in cholesterol-fed rabbits for a limited period. Later the same investigator [1b] reported that choline may cause reabsorption of previously induced atherosclerosis. Morrison and Rossi [2] have reported a similar experience, and several other workers [3] have found choline to be effective in preventing experimental atherosclerosis in rabbits. With this background clinical investigators [4] have used choline in the prevention and the treatment of human arteriosclerosis. Despite this enthusiasm, not all workers have been uniformly successful in demonstrating the beneficial effects of choline in experimental atherosclerosis.[5] Several years ago Pollak [6] emphasized the importance of the age and the weight of rabbits in the production of dietary atherosclerosis. Recently, Firstbrook [7] called attention to the significant effect of changes of body weight on the development of atherosclerosis in rabbits, and

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1. Steiner, A.: (*a*) Proc. Soc. Exper. Biol. & Med. **38**:231, 1938; (*b*) **39**:411, 1938.

2. Morrison, L. M., and Rossi, A.: Proc. Soc. Exper. Biol. & Med. **69**:283, 1948.

3. Morrison, L. M.: Geriatrics **4**:236, 1949. Keston, H. D., and Silbowitz, R.: Proc. Soc. Exper. Biol. & Med. **49**:71, 1942. Broun, G. O.; Andrews, K. P., and Corcoran, P. J. V.: Geriatrics **4**:178, 1949.

4. Herrmann, G. R.: Am. Heart J. **33**:711, 1947. Morrison, L. M.: ibid. **36**:466, 1948. Morrison, L. M., and Gonzales, W. F.: Proc. Soc. Exper. Biol. & Med. **73**:37, 1950. Editorial, J. A. M. A. **140**:368, 1949.

5. Baumann, C. A., and Rusch, H. P.: Proc. Soc. Exper. Biol. & Med. **38**:647, 1938.

6. Pollak, O. J.: Arch. Path. **43**:387, 1947.

7. Firstbrook, J. B.: Science, **111**:31, 1950.

Wilens [8] noted a similar relationship in human autopsy material. The study herein reported was designed to determine the effect of relatively large doses of choline on the production of experimental dietary atherosclerosis and to relate this to changes in body weight.

METHODS

Male albino rabbits of a New Zealand strain were used. All the animals were from a single colony and were between 10 and 12 months of age. Cholesterol was administered by dissolving 5 Gm. of cholesterol in ether and mixing this with a commercial rabbit chow.[9] The ether was evaporated, and rabbits, paired in cages, were fed cholesterol by this technic three times each week. Choline dihydrogen citrate tablets were ground and mixed into the chow daily. No supplementary feedings of vegetable greens were provided during this experiment.

Total cholesterol was determined on ear vein blood each week by using the Liebermann-Burchard reaction. Each animal was weighed twice weekly throughout the course of the experiment. At the conclusion of the experiment all animals were killed by administration of pentobarbital sodium, and the thoracic and abdominal aortas were removed and fixed in formaldehyde solution U. S. P. The entire aorta was stained with sudan IV, and the presence of atherosclerosis was determined by microscopic examination. The atherosclerosis was arbitrarily graded in the sudan-stained gross preparations on a 1 to 4 plus scale. Gross and microscopic examinations of the liver and the kidneys were made in all cases.

Three groups of 10 rabbits each were studied. The animals were kept 2 to a cage, and each pair received 5 Gm. of cholesterol three times a week for six weeks. One group of animals received no choline throughout the experiment, one group received 1 Gm. of choline dihydrogen citrate per rabbit daily for the last five weeks of the experiment, and one group received 4 Gm. of choline per rabbit daily for the last five weeks of the experiment.

OBSERVATIONS AND COMMENT

The data obtained in this experiment are presented in the accompanying table. In the control series (group 1) the average weight initially was 2,992 Gm., and the average weight gain during the six week experiment was 416 Gm. All but 1 rabbit (270) gained weight, and aortic atherosclerosis was definite in 9 of the 10 animals. The average cholesterol rose from a control value of 74 mg. to 1,423 mg. per hundred cubic centimeters.

In the animals fed cholesterol plus 1 Gm. of choline dihydrogen citrate daily (group 2) the average weight initially was 2,560 Gm., but there was an average weight gain during the experiment of only 73 Gm. Although at the conclusion of the experiment the average total cholesterol value was 1,563 mg. per hundred cubic centimeters, 3 rabbits of this group had no discernible aortic atheroma, and in 2 only 1 plus

8. Wilens, S. L.: Arch. Int. Med. **79**:129, 1947.

9. The chow used was that produced by the Ralston Purina Company, St. Louis.

atheromatosis was found. It is interesting to record that in these 5 animals without significant atheromatosis the average weight gain during the six week experiment was 12 Gm.

In the animals fed cholesterol plus 4 Gm. of choline daily (group 3) the average weight initially was 2,478 Gm. and the average weight gain 298 Gm. At the termination of the experiment the average cholesterol

Effects of Six Weeks of Cholesterol-Choline Feeding on the Development of Aortic Atherosclerosis

Rabbit No.	Initial Weight, Gm.	Weight Change, Gm.	Total Cholesterol		Grade of Aortic Atheroma	
			Initial, Mg. per 100 Cc.	Final, Mg. per 100 Cc.		
268	2,200	+1,314	88	1,263	3+	Group 1. Cholesterol feedings only
270	2,876	— 32	74	1,580	3+	
272	3,016	+410	60	1,419	2+	
274	3,138	+116	64	1,114	0	
276	3,008	+584	72	1,405	3+	
278	2,882	+236	31	944	2+	
280	3,234	+314	64	982	2+	
282	3,279	+362	113	1,580	3+	
286	2,992	+662	109	1,432	2+	
294	3,230	+ 94	72	1,345	4+	
Average	2,992	+416	74	1,403		
137	2,352	+101	67	1,703	4+	Group 2. Cholesterol feeding plus 1 Gm. of choline daily
138	2,676	+302	64	1,303	3+	
139	2,222	—104	47	1,214	1+	
140	2,720	0	58	1,419	0	
141	2,684	+ 78	58	1,872	4+	
142	3,458	+ 4	114	2,612	0	
143	2,332	+102	42	398	4+	
144	2,448	+ 96	74	1,262	0	
145	2,142	+ 64	37	1,405	1+	
146	2,703	— 59	43	1,464	2+	
Average	2,560	+ 73	58	1,564		
147	2,878	—216	33	499	0	Group 3. Cholesterol feeding plus 4 Gm. of choline daily
148	2,330	— 86	60	2,145	4+	
149	2,976	+236	71	1,905	4+	
150	2,978	+246	64	1,276	0	
151	2,292	+273	39	1,902	4+	
152	2,358	+292	43	1,598	3+	
153	2,456	+784	64	1,678	4+	
154	2,972	+608	87	1,180	3+	
155	2,344	+236	59	1,509	0	
156	2,988	+500	65	2,209	4+	
Average	2,478	+296	54	1,626		

value was 1,626 mg. per hundred cubic centimeters. Atheroma failed to develop in 3 rabbits of this group. One of these animals (147) lost 216 Gm. in weight; the others gained 246 and 236 Gm., respectively. One rabbit (148) lost 86 Gm. but terminally had a cholesterol value of 2,145 mg. per hundred cubic centimeters and 4 plus aortic atheroma. In both group 2 and group 3 hypercholesteremia developed approximately as rapidly as it did in group 1, the control series.

The slightly decreased incidence of aortic atheroma observed in the choline-treated animals in this series agrees with the observations previously cited. However, despite the comparable total cholesterol values in the control and choline-treated groups, the average weight gain

of 416 Gm. in the control series, compared with an average gain of only 73 Gm. in those receiving 1 Gm. of choline daily, and of 298 Gm. in those receiving 4 Gm. of choline daily, raises the possibility that the variations in atheromatosis may be related to variations in body weight rather than to specific lipotropic activity. The failure of 4 Gm. of choline daily to offer significantly more protection against atherosclerosis than 1 Gm. per day likewise lends support to the view that the changes in atheromatosis may not be solely attributed to the choline administered.

SUMMARY

The administration of choline dihydrogen citrate slightly decreased the incidence of aortic atheromatosis in rabbits with dietary hypercholesteremia. The variation in the development of atheromatosis may be related to changes in body weight rather than to specific lipotropic activity.

THICKNESS OF THE MEDIA OF THE THORACIC AORTA IN RELATION TO AGE

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THIS STUDY was undertaken to collect data concerning the thickness of the media of the thoracic aorta and the calculated cross-sectional area of the media in relation to age.

In a review of the literature we were unable to find any study directly related to this problem. However, a few observations regarding the aorta are helpful in backgrounding the present study. Roy,[1] Yater and Birkeland,[2] Krafka [3] and many others have shown that the elasticity of the aorta decreases with age. In the "Lectures on Pathology" of Aschoff,[4] reference is made to the work of Thoma, Kaufman and others who proved that the inner circumference of the aorta gradually increases with age. Rottino [5] described the four lesions of medial necrosis, and Hass [6] noted a gradual increase in collagen with increasing age. Blumenthal, Lansing and Wheeler [7] demonstrated that calcification of the media is primarily a process of age.

MATERIAL

The material used in this study represents information derived from 335 consecutive necropsies. Intra-arterial embalming had been accomplished, in most

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1. Roy, C. S.: J. Physiol. **3**:125, 1880-1882.
2. Yater, W. M., and Birkeland, I. W.: Am. Heart J. **5**:781, 1930.
3. Krafka, J., Jr.: Am. J. Physiol. **125**:1, 1939; Arch. Path. **29**:303, 1940.
4. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, chap. 6, pp. 131-153.
5. Rottino, A.: Arch. Path. **28**:377, 1939.
6. Hass, G. M.: Arch. Path. **35**:29, 1943.
7. Blumenthal, H. T.; Lansing, A. I., and Wheeler, P. A.: Am. J. Path. **20**:665, 1944.

instances, prior to the time of necropsy. At necropsy the inner circumference of the aorta was measured at the level of the left common carotid artery and recorded in centimeters.

After the aortic circumference had been measured, the entire aorta was placed in Kaiserling's solution no. 1 for from twenty-four hours to three days. In each case a transverse section was then cut from the ascending aorta at a point about 2 cm. above the aortic ring. The piece of tissue so obtained was placed in a 4 per cent solution of formaldehyde overnight and was then embedded in paraffin and cut by the routine method. The same technic was used for all sections.

TABLE 1.—*Age and Sex of Patients in 335 Consecutive Cases in Which Necropsy Was Performed*

Age, Yr.	Cases	Females	Males
0 to 9	10	2	8
10 to 19	10	3	7
20 to 29	15	8	7
30 to 39	22	11	11
40 to 49	48	13	35
50 to 59	72	27	45
60 to 69	76	24	52
70 to 79	50	14	36
80 to 89	30	12	18
90 to 99	2	0	2
Total	335	114	221

TABLE 2.—*Average Circumference of the Thoracic Aorta in Relation to Age*

Age, Yr.	Cases	Average Circumference of Thoracic Aorta, Mm.
0 to 9	10	27.0
10 to 19	9	43.0
20 to 29	15	45.0
30 to 39	22	51.0
40 to 49	49	58.0
50 to 59	72	64.0
60 to 69	76	69.0
70 to 79	50	78.0
80 to 89	30	81.0
90 to 99	2	85.0

These sections were stained with Verhoeff's elastic tissue stain and counterstained with Van Gieson's connective tissue stain. In each case the thickness of the media was measured in three different places on the same section and the average recorded for each case. All these measurements were taken by the use of a micrometer which had been standardized to read in millimeters. It will be noted that the circumference of the aorta was not measured at the place from which the sections were taken. However, this distance was not great and for practical purposes, we believe, the two can be considered as being similar.

RESULTS

As previously stated, the primary purpose of this study was to determine what changes take place in the size of the media of the thoracic aorta in relation to advancing age. In the text which follows, we shall analyze and comment on the data presented in tables 1, 2, 3 and

4 and in graphs *A*, *B* and *C* of the chart. In table 1 we have listed the ages of the patients by decades, from birth to 99 years, with the number of cases and the number of males and females in each age group in the 335 cases studied. Because of the varying and loosely connected structure of the adventitia and because the thickness of the intima is changed so much by atherosclerosis, we excluded these two structures

TABLE 3.—*Average Thickness of the Media of the Thoracic Aorta in Relation to Age*

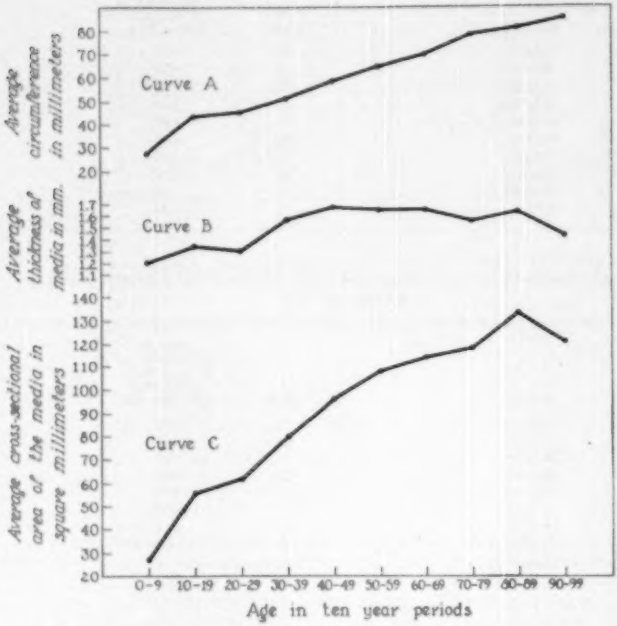
Age, Yr.	Cases	Average Thickness of Media of Thoracic Aorta, Mm.
0 to 9	19	1.21
10 to 19	9	1.34
20 to 29	15	1.30
30 to 39	21	1.56
40 to 49	45	1.67
50 to 59	70	1.65
60 to 69	75	1.65
70 to 79	49	1.55
80 to 89	30	1.62
90 to 99	2	1.43

TABLE 4.—*Average Cross-Sectional Area of the Media of the Thoracic Aorta in Relation to Age*

Age, Yr.	Cases	Average Cross-Sectional Area of Media of Thoracic Aorta, Sq. Mm.
0 to 9	7	27.1
10 to 19	6	53.8
20 to 29	14	62.0
30 to 39	21	80.9
40 to 49	38	96.8
50 to 59	59	106.3
60 to 69	62	114.0
70 to 79	44	118.9
80 to 89	27	133.5
90 to 99	2	121.5

in the measurement of the wall of the aorta. We expected a gradual decrease in the thickness of the media with advancing age, since it is known that the walls of a tubular structure become thinner as the tube dilates. However, it is immediately apparent from the data in table 3 and from curve *B* of the chart that the thickness of the media gradually increases from an average of 1.21 mm., in the age group from birth to 9 years, to 1.67 mm., in the fifth decade, and then remains almost constant from 50 to 69 years. After that time there appears to be a decline. However, the magnitude of this decline is very small and rises again

for the ten year period from 80 to 89 years. Since there were only 2 cases in which the patients were between 90 and 99 years of age, results from this group cannot be considered as significant. Moreover, in table 4 and in curve *C* of the chart there is shown a gradual increase in the cross-sectional area [8] of the aortic media from 27.1 sq. mm., for patients who are in the first decade of life, to 133.5 sq. mm., for those who are 80 to 89 years of age. Again, there were so few cases in which the patients were more than 89 years of age that these cases must be disregarded.



A composite of the information contained in tables, 2, 3 and 4 in which curve *A* represents table 2, curve *B* table 3, and curve *C* table 4.

Since the thickness of the media gradually increases to the age of 50 years, rather than decreases as would be expected with gradual dilatation,

8. The cross-sectional area of the media was calculated by multiplying the circumference of the thoracic aorta by the thickness of the media. In this regard we wish to acknowledge the assistance given by Mr. R. P. Gage, of the section on biometry and medical statistics of the Mayo Clinic.

it is reasonable to draw the conclusion that some substance or substances have been added to the media. Until the age of 50 years there can be no question as to the gradual increase in thickness of the media, and after this age, we believe, measurements of the cross-sectional area indicate that some substance or substances have been added to the media. If nothing were added, one would expect the cross-sectional area to remain the same or to decrease as the circumference becomes greater. As already stated, the cross-sectional area of the media gradually increases from birth to the age of 90 years, and this, together with measurements of the actual thickness, adds credence to the conclusion that gradually, as the aorta ages, some substance is added to the media. A review of the literature reveals that there are many studies to indicate that various substances are deposited in the media in conjunction with the aging process. A few of these substances are elastic tissue, collagen, calcium and mucin. The answer to the question of what substance or substances were added to the media in our cases was not within the scope of this study.

Although it has been proved by others that the circumference of the aorta gradually increases with age, we have also included this measurement in our material. Table 2 and curve *A* of the chart show that the circumference of the thoracic aorta gradually increases with age. In the first decade the average circumference is 27 mm., and this gradually increases until in patients who are between 90 and 99 years of age it is 85 mm.

SUMMARY

In a review of the literature concerning the size of the aorta with advancing age we were unable to find any study in which the thickness of the media of the thoracic aorta had been measured.

Our material represents 335 consecutive necropsies. At the time of necropsy the circumference of the aorta was measured at the level of the left common carotid artery. A microscopic section was taken from the ascending aorta. The thickness of the media of the specimen of aorta was then measured by means of a micrometer. The information derived from these studies is presented graphically in tables 1, 2, 3 and 4 and a chart. Actual measurement of the thickness of the media and of the circumference of the thoracic aorta and calculation of the cross-sectional area of the media indicate that the amount of tissue composing the media increases gradually with advancing age. This is contrary to the expected decrease in thickness which would occur in accordance with the fundamental principles of physics. Since it is known that the circumference of the aorta increases with age, it is assumed that some substance or substances are added to the media as part of the aging process.

Several investigators have unquestionably proved that the circumference of the aorta gradually increases with age. We have measured the thoracic aorta and arrived at the same conclusion.

CONCLUSIONS

The cross-sectional area and the thickness of the media of the ascending aorta increase with advancing age.

The work of others has been confirmed in the finding that the circumference of the ascending aorta gradually increases with advancing age.

CORONARY ARTERY DISEASE

A Comparison of the Rates and Patterns of Development of Coronary Arteriosclerosis in the Negro and White Races with Its Relation to Clinical Coronary Artery Disease

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DIFFERENCES observed in the incidence of coronary artery disease, angina pectoris and acute myocardial infarction between the Negro and white populations have been the subject of much discussion in the medical literature. Comparative observations have led many investigators to conclude that coronary arteriosclerosis is more commonly seen in the white than in the Negro race, the difference of incidence varying between two and four times.[1] Schwab and Schulze [2] attempted to explain the difference by two factors: (1) the shorter longevity of the Negro and (2) an inherent dissimilarity of the cardiovascular system of the Negro. In an analysis of 450 cases of heart disease encountered in Washington, D. C., Hedley [3] showed that there was a greater incidence of hypertensive heart disease in the Negro than in the white population; in 81 necropsies made on 50 white persons and 31 Negroes, coronary arteriosclerosis was demonstrated in 31 white persons and 6 Negroes of comparable ages. He concluded that coronary arteriosclerosis is a less frequent disease in the Negro than in the white population.

Schulze and Schwab [4] emphasized the effects of nervous factors in the development of hypertension, believing that stress and strain incidental to a new civilization were an influential factor. Schwab [5] studied the neurogenic concept further, utilizing a series of vasomotor

From the Laboratories of the Homer G. Phillips and Jewish Hospitals.

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1. Stone, C. W., and Vanzant, F. R.: J. A. M. A. **89**:1473, 1927. Lisa, J. R., and Ring, A.: Arch. Int. Med. **50**:131, 1932. Schwab, E. H., and Schulze, V. E.: Am. Heart J. **7**:223, 1931. Gager, L. T., and Dunn, W. L.: M. Ann. District of Columbia **2**:112, 1933. Holoubek, A. B.: Am. Heart J. **29**:168, 1945.
2. Schwab, E. H., and Schulze, V. E.: Am. Heart J. **7**:223 and 710, 1932.
3. Hedley, O. F.: Pub. Health Rep. **50**:1127, 1935.
4. Schulze, V. E., and Schwab, E. H.: Am. Heart J. **11**:66, 1936.
5. Schwab, E. H.: Proc. Soc. Exper. Biol. & Med. **32**:583, 1935.

tests as outlined by Hines and Brown[6]; he reported that the elevation of blood pressure was more striking in Negroes and concluded that there was a quantitative racial difference in reaction indicating a more sensitive vasomotor mechanism in Negroes. This is supported further by the well known high incidence of arterial hypertension in the latter group, but it fails to explain why this is not associated with more severe coronary arteriosclerosis. There is valid statistical evidence for the greater incidence of hypertension in the Negro race.[7] Moritz and Oldt,[8] in a study of 1,177 autopsies, showed that although Negroes comprised 20 per cent of the subjects, the incidence of hypertension among them was 30 per cent.

The infrequency of coronary thrombosis in Negro patients has been reported by many observers, who have noted a predominance in the white population ranging from four to twelve times.[9] Ashman,[10] on the basis of electrocardiographic studies, concluded that coronary thrombosis is nine times as prevalent in white persons as in Negroes, and the persistence of the juvenile pattern on the electrocardiogram has been noted in a much higher proportion of Negroes than white persons.[11] Burch and Voorhies,[12] in a report from the Charity Hospital, New Orleans, showed the ratio of white to Negro patients having coronary thrombosis and angina pectoris to be 7 to 2 and 4 to 1, respectively. Some comparative studies, however, do not reflect these high ratios. In 2,000 consecutive autopsies equally divided between white persons and Negroes at Louisville General Hospital, Hunter[13] found coronary disease in 16 Negroes and 18 white persons—a ratio of 1:1.1. French and Dock[14] in a selective study of uncomplicated coronary lesions in military personnel between 20 and 36 years of age felt that there were no racial tendencies toward coronary sclerosis. Yater and associates[15] found the incidence of Negro coronary disease to be two-thirds that of white. However, most evidence points to the relative

6. Hines, E. A., Jr., and Brown, G. E.: Ann. Int. Med. **7**:209, 1933.

7. (*a*) Adams, J. M.: Am. J. M. Sc. **184**:342, 1932. (*b*) Thonnard-Neumann, E.: Eighteenth Annual Report of United Fruit Company, sec. **5**:251, 1929, (*c*) Johnston, C.: Am. Heart J. **12**:162, 1936. (*d*) Rowntree, L. G.; McGill, E. H., and Edwards, T. I.: J. A. M. A. **123**:181, 1943.

8. Moritz, A. R., and Oldt, M. R.: Am. J. Path. **13**:679, 1937.

9. Bruenn, H. G.; Turner, K. B., and Levy, R. L.: Am. Heart J. **11**:34, 1936. Levy, R. L., and Bruenn, H. G.: J. A. M. A. **106**:1080, 1936. Peery, T. M., and Langsam, S. M.: Am. Heart J. **19**:424, 1940. Thonnard-Neumann.[7b]

10. Ashman, R.: Tri-State M. J. **13**:2686, 1941.

11. Littman, D.: Am. Heart J. **32**:370, 1946.

12. Burch, G. E., and Voorhies, N. W.: Am. J. M. Sc. **198**:685, 1939.

13. Hunter, W.: J. A. M. A. **131**:12, 1946.

14. French, A. J., and Dock, W.: J. A. M. A. **124**:1233, 1944.

15. Yater, W. M.; Traum, A. H.; Brown, W.; Fitzgerald, R. P.; Geisler, M. A., and Wilcox, B. B.: Am. Heart J. **36**:334, 481, 683, 1948.

infrequency of coronary thrombosis in Negroes, and this is further substantiated by the data obtained in a clinical analysis of 317 cases of heart disease at Hubbard Hospital, Nashville, Tenn., an all Negro institution. In this group there were only 35 cases (11 per cent) of coronary heart disease.[16] Further supporting this observation are the comparative data concerning the death rates per hundred thousand from diseases of the coronary arteries published by the Federal Security Agency[17] (table 1).

In a recently reported study, Lansing, Blumenthal and Gray[18] traced the progressive steps in the genesis of coronary arteriosclerosis in white patients. Their material, consisting of elastic tissue and microincinerated preparations of coronary arteries of persons of various ages, has been made available to us for a comparative study of the rates of development of coronary arteriosclerosis in the white and Negro races. The data concerning coronary arteriosclerosis constitute the primary part of the present report, but comparative figures on the necropsy

TABLE 1.—*Death Rates per Hundred Thousand from Disease of the Coronary Arteries*

	White Population	Negro Population
1940	81.8	24.6
1945	106.6	41.3

incidence of coronary thrombosis and myocardial infarction are also presented. In addition to offering the possibility of an explanation of the differences in incidence of coronary disease in the two races, the present data also bear on the relation of the elastic tissue–calcium changes in the wall and intimal atheromatosis.

MATERIAL AND METHOD

A tabulation of consecutive autopsies from four general hospitals in the St. Louis area was used in establishing the incidence of coronary artery disease. The coronary arteries of 47 Negro patients, 23 males and 24 females, have been studied. Specimens were taken from the anterior descending branch of the left coronary artery within the first 2 cm. of its origin and fixed in formaldehyde solution U. S. P. diluted 1:10 in absolute alcohol. Sections were prepared by the usual paraffin-embedding technic and cut at a thickness of 6 to 8 microns. One section was stained with hematoxylin and eosin; the adjacent section was stained for elastic tissue by the Weigert-Verhoeff method, and a third section was studied by dark field illumination after incineration. The sections were prepared under

16. Thomas, J.: J. A. M. A. **38**:202, 1946.

17. Federal Security Agency, United States Public Health Service, Office of Vital Statistics: Vital Statistics, in Special Report, **27**:295, 1948.

18. Lansing, A. I.; Blumenthal, H. T., and Gray, S. H.: J. Gerontorol. **3**:87, 1948.

the same conditions as those of the coronary arteries of white subjects, and the criteria established by Lansing, Blumenthal and Gray,[18] were followed in this evaluation. These workers collaborated closely with us during the investigative phases of this study.

The ages of the patients concerned in table 2 have been substantiated by birth certificates and insurance contracts. Not included in the group shown in table 2 were 2 patients whose ages were unverified.

The figures set forth in table 3 were obtained from relatively comparable groups. The Jewish Hospital is a private hospital for white patients and, correspondingly, St. Mary's Infirmary is a private hospital for Negro patients; the other two hospitals represent municipal institutions for the two racial groups. The figures in column 4 represent cardiac infarcts of recent or old origin as

TABLE 2.—*Age Distribution of Negroes Studied*

Age Group	Negroes
0-30 yr.	7
31-50 yr.	17
51-70 yr.	16
Over 71 yr.	7
Total	47

TABLE 3.—*A Comparison of the Necropsy Incidence of Coronary Thrombosis and Myocardial Infarction in the Negro and White Races*

Source	Race	Number of Necropsies	Number in Which Coronary Thrombosis Was Shown, With or Without Fresh and Healed Infarcts	Percentage
Jewish Hospital *	White	535	123	23.0
City Hospital †	White	1,496	239	15.9
St. Mary's Infirmary ‡	Negro	207	5	2.4
Homer G. Phillips Hospital §	Negro	2,736	27	1.0

* Private hospital, St. Louis. Data obtained from S. H. Gray.
† Municipal hospital, St. Louis. Data obtained from Dr. J. A. Saxton Jr.
‡ Private hospital, St. Louis. Data obtained from Dr. H. T. Blumenthal.
§ Municipal hospital, St. Louis.

well as recent coronary thrombosis without infarction. It is apparent that there is a considerable difference in the incidence of coronary thrombosis in white and Negro groups.

A COMPARISON OF THE RATES OF DEVELOPMENT OF CORONARY ARTERIOSCLEROSIS IN THE WHITE AND NEGRO RACES

Age Group 0 to 30 Years.—During the first decade of life the coronary artery of the Negro shows a distinct thin unicellular endothelial layer immediately overlying the internal elastica; the latter is well defined, thin, and possesses small graceful undulations. Microincineration demonstrates the unicellular-nuclear calcification of the intima with

no deposition along the internal elastica and a nuclear pattern present throughout the media. This is essentially similar to that present in the corresponding age group of the white population. During the decade between 20 and 30 years the coronary artery of the Negro reveals a distinct quantitative variation as compared with this vessel of the white group. The intima is thickened by proliferation of young fibroblasts to the point of forming 10 to 20 per cent of the thickness of the vessel wall. Minimal deposition of hyalin occurs in the subintimal area immediately adjacent to the internal elastic lamella. The latter is distinct but has a tendency to become straight and is slightly thicker than in the younger vessels in this group (fig. 1 *A*, *a*). Microincineration reveals a thin delicate line of calcium deposited along the inner surface. An average of 1 plus calcification is localized to the position of the internal elastica, with only a nuclear pattern elsewhere in the media (fig. 1 *A*, *b*). A comparison of the coronary arteries of white persons and Negroes shows no significant differences in the degree of calcification up to age 25. At this point the coronary arteries of Negro patients show a lower degree of fragmentation and straightening of the internal elastic lamella, and a somewhat lighter deposit of calcium is present along the elastic fragments.

Age Group 31 to 50 Years.—During the period of 31 to 50 years the intimal layer of the coronary artery of the Negro occupies approximately one-third to one-half the entire wall and is almost equal in thickness to the media. The thickening is diffuse and is due primarily to an increase of hyalin; plaques are encountered only occasionally. This change is, therefore, quantitatively similar to that observed by Lansing, Blumenthal and Gray,[18] who noted an increase of hyalin with loss of fibroblasts in the coronary arteries of white patients. Elastic tissue preparations reveal a straightened, thickened internal elastica with fraying at the edges; small thin splinters of elastic material project into the adjacent subintima and media. In the arteries of Negroes the individual elastic fibers swell diffusely and show relatively little fragmentation (fig. 1 *B*, *a* and *C*, *a*); fragmentation is more pronounced in the coronary arteries of white patients. In the Negro the area adjacent to the internal elastic lamella shows on the average 1 plus calcification, with focal intensification beneath the infrequent plaques. A 2 plus deposition of calcium is noted confined to the few areas of focal fragmentation (fig. 1 *B*, *b* and *C*, *b*).

Age Group 51 to 70 Years.—During the first ten years of this age group the intima of the coronary artery of the Negro occupies approximately one-half the thickness of the vessel wall, with subintimal plaques occurring more frequently and showing hyalinization, cholesterol slits and areas of early calcification. The internal elastic lamella is straight,

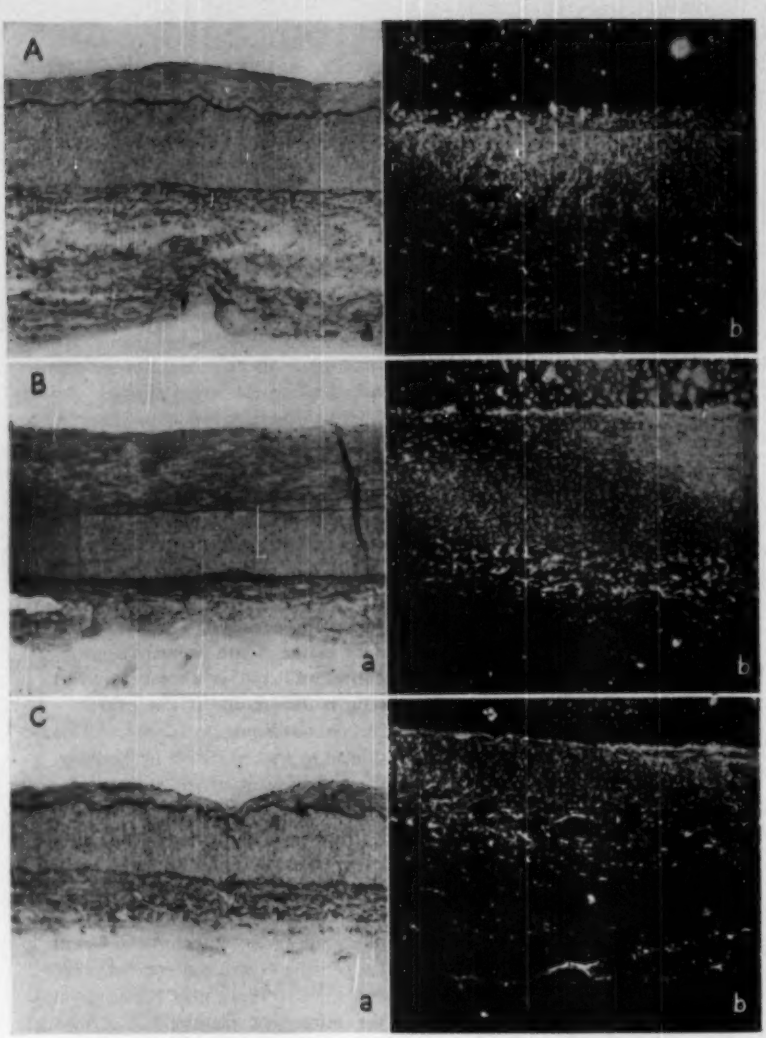

Fig. 1.—Sections of the wall of the anterior descending branch of the left coronary artery (× 85): (*a*) Weigert-Verhoeff stained sections; (*b*) dark field illumination of microincinerated sections.

A, 29 year old Negro woman: (*a*) The internal elastica is well defined, intact, and shows graceful undulations. The subintima is thickened by proliferating fibrous tissue and hyalin. (*b*) Microincineration reveals thin, fine calcium duplication of the elastica.

B, 37 year old Negro man: (*a*) The elastica is straightening and shows very early fragmentation. (*b*) Calcium deposition is 1 plus along the internal elastica, with a nuclear pattern elsewhere.

C, 45 year old Negro woman: (*a*) The fragmentation of the elastica is moderate, with a tendency toward penetration of the subintima. (*b*) Calcification is 1 plus and follows fragments.

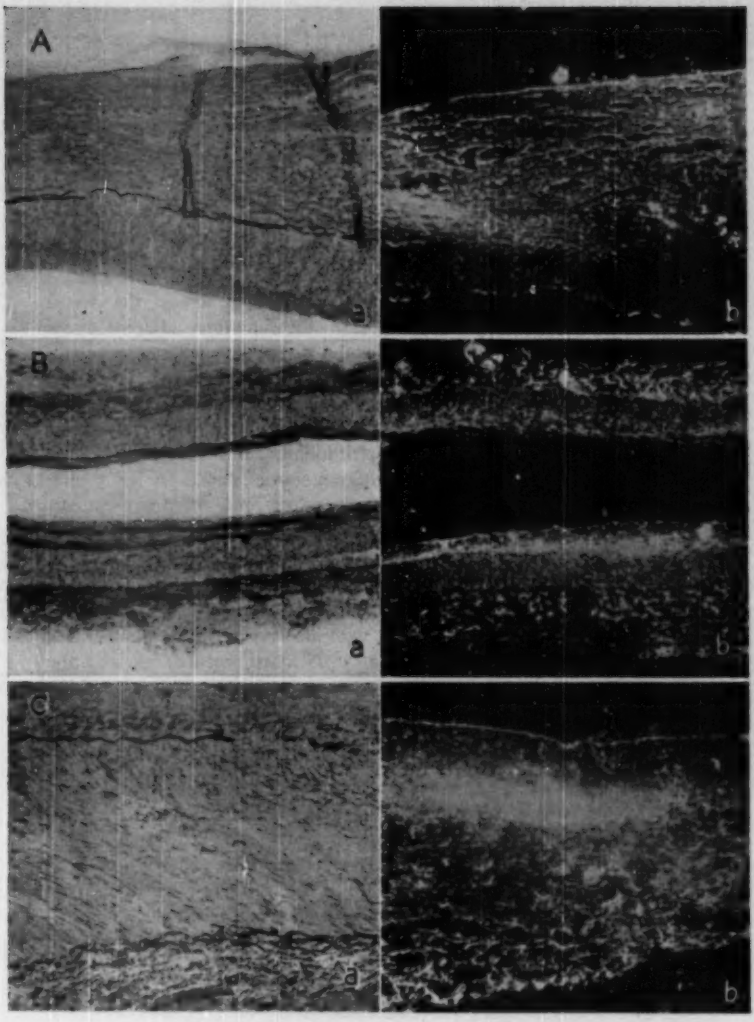

Fig. 2.—Sections of the wall of the anterior descending branch of the left coronary artery (× 85): (*a*) Weigert-Verhoeff stained sections; (*b*) dark field illumination of microincinerated sections.

A, 53 year old Negro man: (*a*) Toward the subintima, fragmentation is more marked, with a point of disruption of continuity. (*b*) The 3 to 4 plus calcium deposition indicates that fragments have migrated into the adjacent subintima.

(Plaques are absent in *B* and *C* despite heavy calcification and pronounced elastic tissue changes.)

B, 67 year old Negro man: (*a*) Fraying is severe, with fragments lying parallel to the elastica. (*b*) The 3 to 4 plus calcification duplicates the elastica and its fragments.

C, 83 year old Negro woman: (*a*) Complete breaks occur in the frayed elastica. (*b*) Heavy 4 plus calcification occurs, extending into adjacent subintima and media.

(For findings in the coronary vessels, of white subjects, the reader is referred to Lansing, Blumenthal and Gray.[18])

thickened, and shows more fraying, especially beneath intimal plaques. Elastic fragments extend into the intima as well as into the media, but are more pronounced in the former direction. No complete break has been noted in the internal elastica, and the bundles of this material have a compact arrangement (fig. 2 *A*, *a* and *B*, *a*). The elastic tissue breakdown and proliferation occurring with age are, therefore, not so severe as those seen in the arteries of white patients.

Calcification of the endothelium is still shown by a fine line. A slightly greater than 1 plus calcium deposition occurs in the deeper portions of intimal plaques as well as in the subintima adjacent to the internal elastica. Fraying and thickening of the internal elastic lamella occur and are accompanied with an increased deposition of calcium slightly greater than 2 plus in intensity. Minimal calcification occurs in the media where fragments of elastic material can be identified. The calcium pattern observed at the base of plaques follows the course of the elastic tissue fragments derived from the internal elastic lamella. A few islands of 4 plus calcification occur in this age group but, when present, are limited to plaques and do not extend into the depths of the media (fig. 2 *A*, *b* and *B*, *b*).

Age Group 71 and Over.—After the age of 71 a progressive hyaline thickening of the intima has resulted in a layer occupying approximately 70 to 80 per cent of the vessel wall, with an increase in the number and the size of the plaques in the subintima. A more profuse fragmentation and spreading of the inner elastica occur in patients over 80 years, giving the impression of numerous small fibrils clumped together, with diffuse scattering through the subintima and media (fig. 2 *C*, *a*).

Calcification of the internal elastic lamella was generally slightly in excess of 3 plus intensity, with numerous patches showing 4 plus calcification at the base of subintimal plaques and occasionally in the media. Maximum calcification (4 plus) occurring in the internal elastica, subintima and media was found rarely during the fifth and sixth decades and with slightly greater frequency up to the eighth decade of life (fig. 2 *C*, *b*). They were found in all subjects over age 80. Such a marked degree of calcification is commonly present in the coronary arteries of white patients after age 50.

COMMENT

These data show that coronary artery disease and coronary thrombosis occur with considerably less frequency in the Negro than in the white population. They also indicate that the rate of development of this degenerative process is slower in Negroes than in white persons. A careful comparison of the arteries of corresponding age groups of the two races leads to the conclusion that the rate of development of coronary arteriosclerosis of the Negro lags behind that of the white person

by approximately a decade. It was noted in this study that this lag is most pronounced during the fourth, fifth and sixth decades, while in other age groups the histologic changes are approximately comparable.

In addition to the foregoing quantitative differences in the development of sclerosis of the coronary arteries of the Negro, there are also certain qualitative differences. Lansing, Blumenthal and Gray,[18] in their studies on the coronary arteries of white persons, showed that the earliest changes consisted in a fraying and fragmenting of the internal elastic lamella, a process during which elastic elements extended into the gradually thickening intima and into the inner half of the media; calcification followed closely the pattern of the fragmented elastic tissue, and both of these processes increased progressively with aging. In the Negro the elastic tissue changes are somewhat different. The process begins with a straightening of the graceful undulating pattern and is associated with a swelling of the entire internal elastic lamella. These initial changes are not associated with any noteworthy deposition of calcium. Subsequently there is a fraying of the edges of the lamella and small fibrils begin to split off. These may enter the subintimal region or the adjacent media, or they may remain close to the parent elastic membrane, oriented parallel to it. In the coronary arteries of the oldest age group, in which the latter process has progressed to an impressive degree, there is again little difference between the coronary arteries of Negro and white patients. Calcification of elastic elements occurs at about the time the fraying of the edges of the internal elastic membrane is noted, and, as in the arteries of white patients, becomes more obvious as the fraying and fragmentation increase.

An important conclusion may be drawn concerning the relation of these elastic tissue and calcium changes to the formation of intimal plaques. In studies on the aorta, Blumenthal, Lansing and Wheeler [19] demonstrated that calcification of the media preceded the formation of intimal plaques and concluded from their observations that the medial changes condition the formation of the intimal elevations. They reviewed older evidence supporting such an interpretation. Our observations strongly support the concept of medial calcification preceding the formation of intimal plaques.

Any attempt to explain the origin of these racial differences at the present time would lead only to unsupported speculation. However, it would be well to point out that during the course of this investigation a number of coronary ostiums of Negroes were noted to be situated above the line of closure of the aortic valve cusps. This anatomic factor, if proved significant in comparative studies now in progress, might offer

19. Blumenthal, H. T.; Lansing, A. I., and Wheeler, P. A.: Am. J. Path. **20**:665, 1944.

an explanation. An attempt was also made to discover further unusual anatomic features by injecting hearts according to the technic described by Schlesinger[20]; none was found, nor were any missed infarcts discovered.[21]

These observations, therefore, offer an explanation for the low incidence of coronary thrombosis and myocardial infarction in the Negro race. Other arteries are being studied and compared with those of white patients in order further to characterize differences in the pattern and the rate of arteriosclerosis and to attempt to correlate these with differences in the clinical manifestations of vascular diseases in the two races.

SUMMARY

These investigations show that there is a lower incidence of coronary thrombosis in the Negro than in the white population despite the higher incidence of arterial hypertension in the former. A ten year lag of the onset of changes of coronaries due to aging occurs in the Negro; this delay assumes significance during the fourth, fifth and sixth decades. Studies based on elastic tissue staining and microincineration technics show that the elastic elements of the arteries of Negroes manifest a greater tendency to swell but a lesser tendency to fragment and calcify than do the corresponding elements of the coronary arteries of white persons. These observations tend to substantiate the concept that the formation of intimal atheromatous plaques is conditioned by the severity of the elastic tissue changes and the subsequent calcification of this tissue.

20. Schlesinger, M. J.: Am. Heart J. **15**:528, 1938.

21. Sclafford, U. M.: Personal communication to the authors.

TRICHOMA: TUMOR OF HAIR ANLAGE

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CELLS of the epidermis capable of cell division are located, according to Thuringer,[1] in the basal layer and in the adjacent spinous layer of the epidermis. They are also present in structures derived from downgrowths of the basal layer, such as hair follicles, sebaceous glands, sweat glands and apocrine glands. The ectodermal ancestor cell of the basal layer is capable of differentiating into any one of the structures mentioned and may give rise, therefore, to any epithelial neoplasm of the skin. Most of the epithelial cancerous growths of the skin mimic the pattern of the epidermis and are diagnosed as non-keratinizing or keratinizing squamous cell carcinoma. Rarely they imitate the structure of sebaceous glands, sweat glands or apocrine glands. Practically all the benign growths mimic the pattern of skin appendages and are named accordingly sweat gland, sebaceous gland or apocrine gland adenoma; some imitate the structure of hair follicles. Recently the neoplasms imitating the structures of skin appendages were thoroughly investigated by Gates, Warren and Warvi,[2] Foot[3] and Lever.[4] While there is considerable agreement as to the nature of most of these growths, the cellular origin of the commonest of the group, the so-called basal cell tumor, remains under discussion. The cellular structure and behavior of this growth suggest that it does not arise, as the name implies, directly from the basal cells of the epidermis but rather from cells already differentiated to form hair follicles. The present study seeks to substantiate the hair matrix or anlage origin of these growths.

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Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

1. (*a*) Thuringer, J. M.: Anat. Rec. **29**:31, 1924; (*b*) **40**:1, 1928. (*c*) Cowdry, E. V., and Thompson, H. C.: ibid. **88**:403, 1944. (*d*) Thuringer, J. M., and Cooper, Z. K.: ibid. **106**:89, 1950.

2. Gates, O.; Warren, S., and Warvi, W. N.: Am. J. Path. **19**:591, 1943.

3. Foot, N. C.: Am. J. Path. **23**:1, 1947.

4. Lever, W. F.: Arch. Dermat. & Syph. **57**:679 and 709, 1948.

METHOD

Microscopic preparations of 100 neoplasms diagnosed as basal cell tumors were taken from the files of the department of pathology of Baylor University College of Medicine and of the laboratory service of the Veterans Administration Hospital. These were regarded as constituting a random sample of such growths. An attempt was made to evaluate in routine microscopic preparations the individual and group characteristics of the component cells and the relation of the cells to the stroma. This was followed by a detailed study of the neoplasms to detect clues to their cellular origin. In addition a special study was made of 16 neoplasms derived from 2 patients. With these exceptions, data were not assembled on site, age, race and sex incidence; nor were the duration and the clinical course and response to treatment correlated with the microscopic structure of the growths.

MICROSCOPIC STUDY

The preliminary survey disclosed that the growths studied represented various stages of development of the neoplasms. Some were incipient and were covered by fairly intact epidermis, others were partly devoid of covering epithelium, and again others (about 20 per cent) disclosed evidences of having been exposed to radiation or other forms of treatment. Even in these the neoplastic cells retained their individual and group characteristics and changed only their relation to the epidermis and to the corium (fig. 1 *A*). The cellular patterns of the individual growths had many features in common. On the other hand, there were many dissimilarities which account for the various names that have been applied to these neoplasms.

Most instructive for our purposes were the growths in their incipient state. At this stage, while the cellular patterns varied considerably, their relations to the epidermis and the corium were almost constant. Anywhere in the corium beneath the epidermis and proximal to the roots of hair follicles, small or larger groups of neoplastic cells formed rosette-like structures (fig. 1 *B*). The neoplastic cells had a streamlike, radiating or concentric pattern with palisading of the peripheral cells. The cell nuclei were round or oval, mostly compact, and deeper stained than the cells of the epidermis or of nearby hair follicles. Their cytoplasm was scanty or barely perceptible. Intercellular bridges could not be made out. The cell nests were solid or formed a lacelike pattern with anastomosing cell strands (fig. 2 *A*). In some growths, in the center of large cell nests there were areas of liquefaction or necrosis (fig. 2 *B*). In other growths a concentric pattern could be made out, with keratinized centers eccentrically located within the cell nests (fig. 3). Each of these keratinized centers resembled a hair follicle imperfectly formed, or at times possibly represented a hair follicle caught in the growth. In some growths the lacelike pattern was produced by liquefaction within the cell nests. The spaces or pseudolumens gave such growths a glandular appearance. Palisading of the peripheral cells

Fig. 1.—*A*, trichoma, tumor of hair anlage (× 60), from the face of a 51 year old white man. The growth invaded the sclera and necessitated removal of the eyeball. In cell nests the neoplastic cells have a whorled streamlike arrangement, with the center mimicking the pattern of a hair follicle.

B, tumor of hair anlage (× 60), from the neck of a 65 year old white man (J. J. K.). He had several lesions over the scalp and one on the right frontal area and another on the lumbar area. The neoplastic cell nests form rosette-like structures with palisading of the peripheral cells.

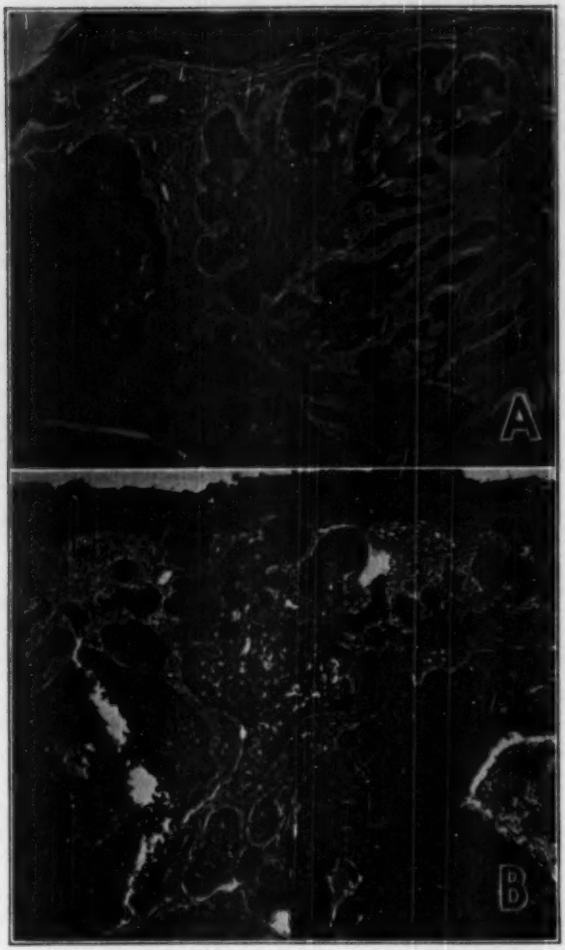

Fig. 2.—*A*, tumor of hair anlage (× 60), from the left temporal region of a middle-aged white man. Some of the cell nests are solid, and others form a lacelike pattern with anastomosing cell strands.

B, tumor of hair anlage (× 60), from the forehead of a 57 year old white man. In the centers of the large cell nests there are areas of liquefaction or necrosis.

usually was a conspicuous feature, though not present in all growths (fig. 4). The cells of occasional neoplasms contained brown or dark brown pigment granules. The number of cells in a state of division

Fig. 3.—*A*, tumor of hair anlage (× 60), from the right malar region of a 34 year old white man (R. A.). From him nine other lesions were removed. The concentric pattern with keratinized centers eccentrically located resembles imperfectly formed hair follicles.

B, tumor of hair anlage (× 60), from the inner canthus of the right upper eyelid of a 65 year old white man. The concentric pattern with imitation of hair follicles is even more clearly seen than in *A*.

varied in different growths. Cell division appeared to occur mostly near the palisading layer.

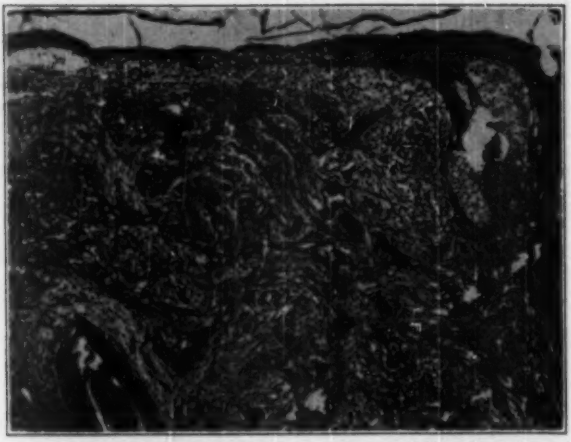

Fig. 4.—Tumor of hair anlage (× 60), from the right cheek of a 45 year old white man. The neoplastic cell nests are in anastomosing strands with no palisading of the peripheral cells.

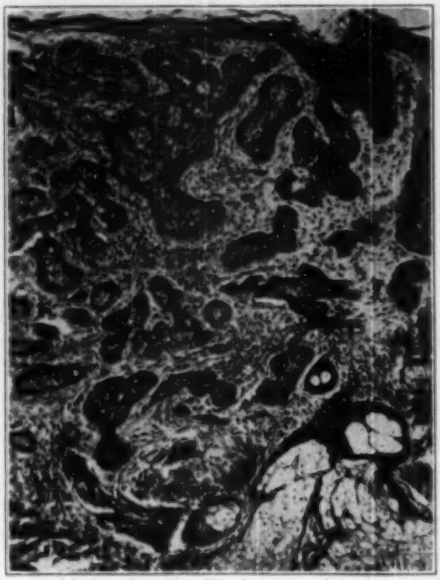

Fig. 5.—Tumor of hair anlage (× 60), from the right frontoparietal region of J. J. K. The neoplastic cell nests seem not to disturb a nearby hair follicle with its sebaceous glands and arrector pili muscle.

The neoplastic cell nests were within a scanty or more abundant, loose, fibrillar, newly formed connective tissue stroma. In the apparently incipient growths the other structures nearby in the corium, such as hair follicles with their musculi arrectores pilorum, sebaceous glands and sweat glands, appeared undisturbed (figs. 5 and 6). These structures were usually not present within the neoplastic fields. Even the thickness of the overlying epithelium retained its usual width, with the epithelial ridges discernible. There appeared to be no, or only

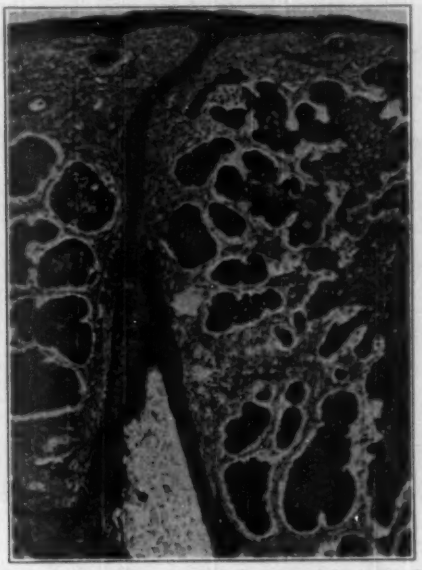

Fig. 6.—Tumor of hair anlage (×60), from the left cheek of a 56 year old white man. The neoplastic cell nests are grouped at either side of a hair follicle. At the right beneath the epidermis there is the opening of a sweat gland.

occasional, connections with either the basal layer of the epidermis or the adjacent hair follicles. Other growths that were apparently in a further stage of their evolution obviously interfered with the course of hair follicles and fused with the sheaths of these structures. The epidermis overlying such growths was thin, with no epithelial ridges. Over such areas the basal layer was indistinct and the spinous layer was narrowed, with corresponding thinning of the granular and keratinized layers. In other growths the neoplastic cells touched the epidermis (fig. 7) or adjacent hair follicles. There was, however, no

proliferative change in the nearby cells. Therefore, these relations appeared to be accidental rather than essential. All the characteristic features of this type of growth could be well illustrated in the multiple lesions of the two patients mentioned under "Method." In the case of patient R. A., a 34 year old white man, specimens were obtained from lesions of the left upper eyelid, the upper part of the back, the right supraorbital region, the lower eyelid, the malar region (fig. 3*A*), the temple, the neck (fig. 7), the deltoid region and the middle part of the back. In the case of patient J. J. K., a 65 year old white man, specimens were obtained from several lesions of the scalp, the right frontal area (fig. 5), the neck (fig. 1*B*) and the lumbar area. Frequently the neoplastic cell nests appeared at various distances from one another as

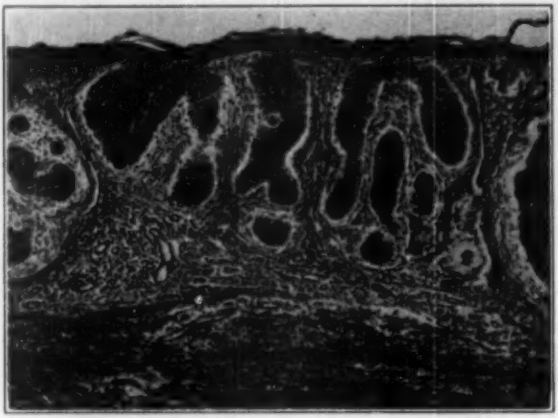

Fig. 7.—Tumor of hair anlage (× 60), from the neck of R. A. The neoplastic cell nests touch the epidermis without producing changes in the nearby cells.

if they were of multicentric origin. This was particularly true in the lesions of the 2 patients cited.

In growths with destruction of the covering epidermis and ulceration the neoplastic cells were exposed. In these the extension of the neoplasm appeared to be lateral and rather superficial and was deepest opposite the exposed and ulcerated surface. Only in such growths, usually those of long standing, did the neoplastic infiltration extend below the level of hair follicles.

COMMENT

A number of features distinguished the neoplasms called basal cell tumors. These growths are advisedly not called carcinoma, because, though locally invasive, they do not involve the regional lymph nodes

nor do they produce distant metastases. There is no more justification to mark as cancerous a patient with a basal cell tumor than one with an ameloblastoma. This understanding is not new,[5] and is opposed only by those who evaluate the behavior of these neoplasms by the destructive effects of the growths that are neglected or maltreated.

The original concept of Krompecher[6] that these neoplasms arise from the basal layer of the epidermis, as implied in the name, is not well substantiated. The basal layer of the epidermis serves as the delimiting layer of the epithelium toward the corium, and aids in the recovering of denuded surfaces. It is common experience that repeated attempts by surface epithelial cells to cover a denuded area of skin do not result in a basal cell tumor. When cancer develops in such an area of chronic ulceration, it is found to be squamous cell carcinoma. Furthermore, basal cell tumors appear only at sites where skin appendages are present, and they commence as nodules in the corium. They do not occur at sites covered by squamous epithelium where skin appendages are lacking. They have not been observed in the oral cavity, pharynx or esophagus, nor in the vagina, cervix uteri, etc., nor have they been reported on the soles or the palms or on parts of the fingers or the toes where hair follicles are wanting and where sweat glands are abundant. They occur most frequently on or adjacent to exposed pileous areas of the skin. This lends support to the suggestion made by Mallory[7] and also by Haythorn[8] that these neoplasms are derived from hair follicles or from their anlages.

The hair follicles are ectodermal structures, and associated with them are sebaceous glands and the musculi arrectores pilorum. It is our impression that the so-called basal cell tumor arises either from cells of the matrix differentiated to form the hair follicle or from the anlage of the hair follicle that did not fully develop and that lacked concomitant sebaceous glands and the arrector pili muscle. The facts that the neoplasm in its incipient stage is located in the corium between the epidermis and the level of the roots of hair follicles, that it extends laterally and that it tends to grow toward the epidermis as do hairs rather than toward the subcutis are all in favor of this concept. The variation in pattern depends on the degree to which the growth imitates the structure of a hair follicle. The name "trichoma" is suggested as an appropriate designation for these growths originating from hair matrix or anlage.

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5. Wallace, S. A., and Thomas, J. R.: Texas State J. Med., to be published.
6. Krompecher, E.: Der Bzsalzellenkrebs, Jena, Gustav Fischer, 1903.
7. Mallory, F. B.: J. A. M. A. **55**:1513, 1910.
8. Haythorn, S. R.: Am. J. Cancer **15**:1069, 1931.

SUMMARY AND CONCLUSIONS

A microscopic study is presented of a random sample of 100 so-called basal cell tumors. The structural features, the sites of occurrence and the behavior of these growths favor the assumption that they arise either from cells of the matrix differentiated to form the hair follicle or from the anlage of the hair follicle rather than directly from the basal cells of the epidermis. "Trichoma" is suggested as an appropriate designation for these growths.

SKELETAL CHANGES IN SEVERE PHOSPHORUS DEFICIENCY OF THE RAT

I. Tibia, Metacarpal Bone, Costochondral Junction, Caudal Vertebra

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CONSIDERABLE interest has been shown in the past in the effects of phosphorus and vitamin D deficiencies in experiments inducing rachitic changes in the skeletal system of the rat. Since the earlier experimental work of McCollum, Simmonds, Shipley and Park,[1] Sherman and Pappenheimer[2] and Steenbock and Black,[3] rickets has been believed to be due to a disproportion of calcium and phosphorus intake in the absence of vitamin D. The disproportion resulted in a disturbance of the normal process of calcification in the active growing period. It should be noted that in those earlier experiments the effects may have been complicated by vitamin deficiencies not understood at that time. Furthermore, most of the histologic studies were limited for the main part to only one bone—the rib, the femur or the tibia.

Some of the essential data on earlier experimental rickets are given in table 1. In 1921 Sherman and Pappenheimer [2] described a rachitogenic diet which was deficient in phosphorus and in vitamins A and D. At the same time McCollom, Simmonds, Shipley and Park [1] reported that they had produced rickets in rats with a similar diet (McCollum diet 3127). The phosphorus content of this diet was slightly higher

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1. McCollum, E. V.; Simmonds, N.; Shipley, P. G., and Park, E. A.: J. Biol. Chem. **47**:507, 1921.

2. Sherman, H. C., and Pappenheimer, A. M.: J. Exper. Med. **34**:189, 1921.

TABLE 1.—*Data Concerning Experimental Production of Rickets*

Experimental Diet	Author of Histologic Analysis	Year	Animals Used	Age at Beginning of Exper., Days	Duration of Exper., Days	Age at Autopsy, Days	Composition of Diets					Bones Studied
							Ca, %	P, %	Ca:P Ratio	Vitamin A per Kg. of Diet	Vitamin D per Kg. of Diet	
Sherman-Pappenheimer diet 84	Sherman and Pappenheimer *	1921	30	28-29	30-32	58-60	0.550	0.057	6.3:1	"Deficient"	"Deficient"	Rib
McCollum-Simmonds-Shipley-Park diet 2127	McCollum and co-workers †	1921-1922	5	25	24-30	49-54	0.8	0.200	3.8:1	"Deficient"	"Deficient"	Femur
McCollum-Simmonds-Shipley-Park Diet 2133	McCollum and co-workers	1921-1922	6	16	35-98	51-114	0.8	0.200	3.8:1	"Not sufficient"	"Deficient"	Femur
McCollum-Simmonds-Shipley-Park diet 3143	McCollum and co-workers	1921-1922	9	28-50	43-76	80-116	1.25	0.301	4.0:1	"Decidedly low"	"Deficient"	Femur
Steenbock-Black diet 2965	Dodds and Cameron *	1925	32	28	7-56	35-84	1.25	0.250	5.0:1	"Low amount"	"Deficient"	Tibia
Day-McCollum diet 76	Follis, Day and McCollum *	1939	73	21	46-67	69-88	0.40	0.017	23.6:1	15,000 U. S. P. units	2,800 U. S. P. units of D2‡	Tibia and femur
Group 1†		1940	10	21	24	45	0.46	0.013	35:1	20,000 U. S. P. units	8,000 U. S. P. units of D3‡	Tibia, third metacarpal, costochondral junction, 9th caudal vertebra, incisors, molars temporomandibular joint
Group 2†	Present	1940	15	21	22	53	0.43	0.015	29:1	20,000 U. S. P. units	8,000 U. S. P. units of D3‡	
Group 3†	studies	1940	10	21	41	62	0.43	0.005	79:1	20,000 U. S. P. units	8,000 U. S. P. units of D3‡	
Group 4†		1940	14	21	49	70	0.43	0.013	31:1	20,000 U. S. P. units	8,000 U. S. P. units of D3‡	

* D2 is calciferol, an activated plant sterol (viosterol [irradiated ergosterol]).
† For additional information concerning diets see table 3.
‡ D3 is an activated animal sterol (irradiated 7 dehydrocholesterol).

than that of the diet used by Sherman and Pappenheimer. McCollum diet 3133 and the diet of Steenbock and Black [4] contained more butter fat, and this provided a small amount of vitamin A, which prevented the development of vitamin A deficiency symptoms. Their rats also lived longer and had more intense rickets. More severe rickets was also produced when more calcium was added to the diet (McCollum diet 3143). It was concluded that within certain limits the ratio of calcium to phosphorus was of greater importance than the absolute amounts of these minerals in the diet.

Shohl, Brown, Chapman, Rose and Saurwein [5] showed that the growth of rats fed the Steenbock-Black diet was curtailed by 30 to 40 per cent. The addition of vitamins A and D did not promote growth but did prevent the development of rickets. They believed that the limiting factor was the inadequate amount of phosphorus in the diet. Lilly, Pierce and Grant [6] reported mineralization of the rachitic rat skeleton when phosphate was added to their high calcium–low phosphorus diet.

Since the amount of nutritionally available phosphorus was not given for these earlier experimental diets, the calculated calcium-phosphorus ratios as given in table 1 are only estimates. No numerical values for the amounts of vitamins A and D were given, the diets being described simply as "decidedly low," "not sufficient" and "deficient" in vitamins A and D.

Day and McCollum [7] were the first to report the amounts of vitamins A and D in the diets. The phosphorus content was extremely low (0.017 per cent) with a calcium-phosphorus ratio of 23.6:1. In spite of the presence of vitamins A and D, normal bone did not form. Follis, Day and McCollum [8] reported that in these rats the histologic bone changes were qualitatively similar to the classic high calcium and low phosphorus rickets described by McCollum, Simmonds, Shipley and Park,[1] Sherman and Pappenheimer [2] and Dodds and Cameron.[9] Mineral balance studies revealed that a significant amount of phosphorus was mobilized from the bones. Their work indicated that the phosphorus content of the diet was of greater importance than the contents of

4. The diet of Steenbock and Black [4] later became the U. S. P. rachitogenic test diet.

5. Shohl, A. T.; Brown, H. B.; Chapman, E. E.; Rose, C. S., and Saurwein, E. M.: J. Nutrition **6**:271, 1933.

6. Lilly, C. A.; Pierce, C. B., and Grant, R. L.: J. Nutrition **9**:25, 1935.

7. Day, H. G., and McCollum, E. V.: J. Biol. Chem. **130**:269, 1939.

8. Follis, R. H. Jr.; Day, H. G., and McCollum, E. V.: J. Nutrition **20**:181, 1940.

9. Dodds, G. S., and Cameron, H. C.: Am. J. Anat. **55**:135, 1934.

vitamins A and D. The addition of 4.5 per cent of phosphoric acid U.S.P. to the diet was sufficient to restore the process of normal ossification.

A study was undertaken to test phosphorus deficiencies even more severe than those studied by Day and McCollum and to investigate their effects on the skeleton of the rat. Since normal standards for histogenesis of various bones of the rat of the Long-Evans strain had been established in this laboratory for different age groups,[10] it was felt that a histologic study of more than one bone, including the tibia, the third metacarpal bone, the costochondral junction and the ninth caudal vertebra, would be a distinct advantage over previously reported histologic studies.[11]

MATERIAL AND EXPERIMENTAL ARRANGEMENT (TABLE 2)

Forty-nine female rats of the Long-Evans strain, weighing 45 to 50 Gm. each, were weaned at 21 days of age. Twenty-one rats were fed a phosphorus-deficient diet, 20 were pair-fed a phosphate-supplemented diet, and 8 were offered this control diet ad libitum. Food consumption and weight were recorded regularly. The experimental animals were arranged in four groups according to age and duration of the deficiency.

Group 1 were killed at 45 days, group 2 at 53 days, group 3 at 62 days and group 4 at 70 days of age after experimental periods of twenty-four, thirty-two, forty-one and forty-nine days, respectively. The protein of the diet consisted of 20.0 per cent washed beef blood fibrin, which Jones[12] has shown to compare favorably with casein for nutrition. The very low phosphorus content was attained by employing isoelectrically precipitated fibrin for protein, and purified phosphorus-free components for the balance of the diet. The phosphorus contents of the diets are shown in table 2. All the diets were decidedly lower in phosphorus than any previously reported. The formula of the deficient diet is given in table 3.

The experimental as well as the control rats received 8 U.S.P. units of vitamin D and 20 U.S.P. units of vitamin A per gram of diet. The phosphorus-deficient and the pair-fed control rats consumed between 5.2 and 6.5 Gm. of diet per day. They therefore ingested 41 to 52 U.S.P. units of vitamin D and 104 to 130 U.S.P. units of vitamin A daily. The rats fed ad libitum consumed approximately 15 Gm. per day with an intake of 120 U.S.P. units of vitamin D and 300 U.S.P. units of vitamin A. Rats restricted to the phosphorus-deficient diets gained less than 10 Gm. of weight during the first three weeks. They then gradually lost weight and died after seven to eight weeks. A tibia, a metacarpal bone, a costochondral junction and a caudal vertebra were selected for histologic study. The tibia was chosen because of its use as a rachitic standard and because of the late closure of its proximal epiphysial cartilage. The metacarpal bone provided an early closing epiphysial cartilage and had the additional advantage of being a small bone which could be sectioned in its entirety. The costochondral junction

10. (*a*) Becks, H.; Simpson, M. E., and Evans, H. M.: Anat. Rec. **92**:109, 1945. (*b*) Becks, H.; Asling, C. W.; Collins, D. A.; Simpson, M. E.; Evans, H. M.: ibid. **100**:577, 1948. (*c*) Unpublished data.

11. The skulls and teeth will be described in a second paper.

12. Jones, J. H.: J. Nutrition **17**:601, 1939.

is of interest because of the importance of the rachitic rosary in rickets, and the caudal vertebra is used as a standard in growth hormone studies.[13]

TABLE 2.—*Experimental Arrangement*

Group	Treatment	Animals	Average Daily Diet Consumed, Gm.	Age at Beginning of Exper. Diet, Days	Duration of Exper., Days	Age at Autopsy, Days	Composition of Diet: Ca, %	P, %	Ca:P Ratio	Average Daily Intake of Vitamin A, U. S. P. Units	Average Daily Intake of Vitamin D, U. S. P. Units
1	Phosphorus-deficient	5	6.3	21	24	45	0.46	0.013	35:1	130	33
	Pair-fed	5	6.3	21	24	45	0.46	0.475	1:1	130	33
2	Phosphorus-deficient	5	6.0	21	30	51	0.43	0.015	29:1	130	48
	Pair-fed	5	6.0	21	30	51	0.43	0.475	.90:1	130	48
	Ad libitum	5	15.0	21	30	51	0.43	0.475	.90:1	300	120
3	Phosphorus-deficient	5	5.2	21	41	62	0.43	0.005	78:1	104	41
	Pair-fed	5	5.2	21	41	62	0.41	0.53	.81:1	10.	41
4	Phosphorus-deficient	6	6.9	21	49	70	0.45	0.013	31:1	130	46
	Pair-fed	5	6.6	21	49	70	0.43	0.50	.86:1	130	46
	Ad libitum	3	15.0	21	49	70	0.43	0.50	.86:1	300	120

TABLE 3.—*Composition of the Experimental (Phosphorus-Deficient) Diet**

Purified beef blood fibrin	20.0%
Sucrose	20.0%
Hydrogenated cottonseed oil (crisco)§	10.0%
Fish liver oil (400 U. S. P. units vitamin D and 1,000 U. S. P. units vitamin A per gram)	2.0%
Sodium chloride	1.0%
Potassium chloride	1.5%
Magnesium sulfate.7 H_2O	0.5%
Ferric citrate	0.1%
Calcium carbonate	1.0%
Vitamin mixture†	1.0%
Trace element mixture‡	1.0%

* In the control diet the 1.0 per cent sodium chloride is replaced with 2.4 per cent disodium and phosphate.

† Vitamin mixture (per 100 Gm. of diet)

Thiamine hydrochloride	1.0 mg.
Riboflavin	1.0 mg.
Pyridoxine	0.5 mg.
P-aminobenzoic acid	1.0 mg.
Nicotinic acid	5.0 mg.
Calcium pantothenate	5.0 mg.
I-inositol	20.0 mg.
Choline chloride	100.0 mg.
Sucrose	866.5 mg.
	1.0 Gm.

‡ Trace element mixture (per 100 Gm. of diet)

$CuSO_4.5H_2O$	13.0 mg.
$MnSO_4$	4.0 mg.
$ZnSO_4.7H_2O$	2.0 mg.
Cocoatate	1.0 mg.
KI	0.8 mg.
Sucrose	979.2 mg.
Total	1.0 Gm.

§ Registered trademark.

The bones were fixed in 4 per cent neutral formaldehyde solution, roentgenographed and measured. They were then decalcified in 5 per cent nitric acid, embedded in pyroxylin U.S.P. (nitrocellulose), sectioned and stained with hematoxylin and eosin. Measurements of the width of the epiphysial cartilage were made with an ocular micrometer.

13. Freud, J.; Levie, L. H., and Kroon, D. B.: J. Endocrinol. **1**:56, 1939.

ROENTGENOLOGIC OBSERVATIONS

The roentgenogram of the carcasses of the phosphorus-deficient rats revealed an extreme lack of mineralization (fig. 1 *A*). The only well

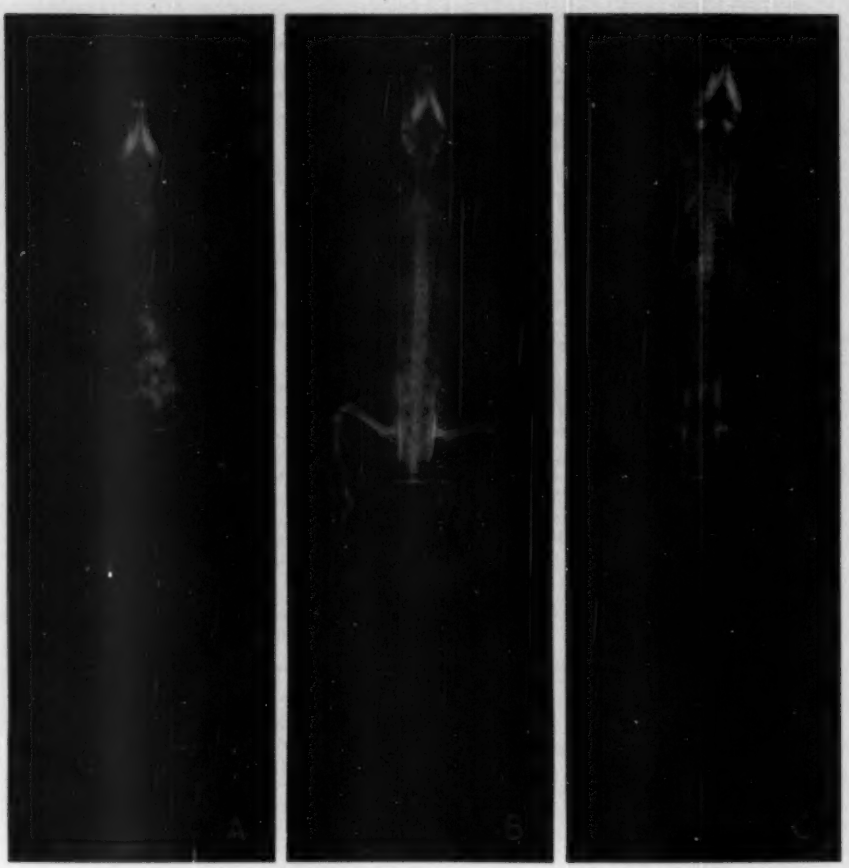

Fig. 1.—Roentgenograms of rat carcasses (half natural size). The first rat had been fed the experimental (phosphorus-deficient) diet for thirty-two days; the second, this diet supplemented with phosphate (control diet); the third, the control diet ad libitum. All were 53 days of age at autopsy. *A*, phosphorus-deficient rat. *B*, pair-fed control. *C*, ad libitum–fed control.

mineralized structures observed in this roentgenogram were the teeth, which were already calcified prior to the beginning of the experiment. Growth was severely stunted.

In figure 1 *B* and *C* are reproductions of roentgenograms of a pair-fed control and an ad libitum–fed control rat and show normal mineralization.

In each of the four groups the average tibia length of the phosphorus-deficient rats (table 4) was less than that of their pair-fed and ad libitum controls. Roentgenographic reproductions of the tibia twice natural size are given in figure 2 *A* for comparison. The diameter of the shaft of deficient tibia (fig. 2 *A*, *a*) at its midpoint and the width of the cortical bone are less than those observed in the pair-fed rats. The deficient rats disclose a club-shaped epiphysis and an abrupt junction with the diaphysis which are characteristic of severe rickets. In contrast to this, the epiphyses of the pair-fed and ad libitum rats taper gradually into the diaphysis. The epiphysial cartilage is very wide, and in the metaphysis the trabeculae are poorly mineralized. In the tibias of the pair-fed and ad libitum controls (fig. 2 *A*, *b* and *c*) the width of the epiphysial cartilage is narrow and the trabeculae are well ossified and distinctly seen.

The roentgenograms of the forepaws of the phosphorus-deficient rats (fig. 2 *B*, *a*) when compared with those of their controls (fig. 2 *B*, *b* and *c*) show similar changes, viz., a lack of mineralization, shorter and narrower bones, and an increase of width in the nonmineralized epiphysial cartilage and metaphysis. The forepaws of the ad libitum and pair-fed control rats are well developed and mineralized and the width of the epiphysial cartilage appears normal.

A roentgenogram of the ninth caudal vertebra of a phosphorus-deficient rat (fig. 2 *C*, *a*) reveals the characteristic roentgenographic appearance of rickets. As compared with that of the pair-fed and that of the ad libitum control, the maturation of the vertebra is severely retarded. In both the proximal and the distal epiphysis the secondary ossification centers appear as two small radiopaque areas. In the pair-fed and ad libitum controls these centers have united and form the completed epiphysis (fig. 2 *C*, *b* and *c*). The lack of growth of the ninth caudal vertebra of the deficient rats is marked. The vertebrae of the pair-fed control rats are smaller than the ad libitum controls because of the limited food intake.

HISTOLOGIC OBSERVATIONS

The histologic aspects of all bones of the ad libitum control groups compare favorably with the standards established in this laboratory for the Long-Evans strain rats at various age levels.[10]

The proximal epiphysial region of the tibia of rats fed ad libitum is shown histologically in figure 3. The epiphysial cartilage has an average width of 240 and 186 microns in groups 2 and 4, respectively (table 4). The cells of the vesicular zone are large; numerous long,

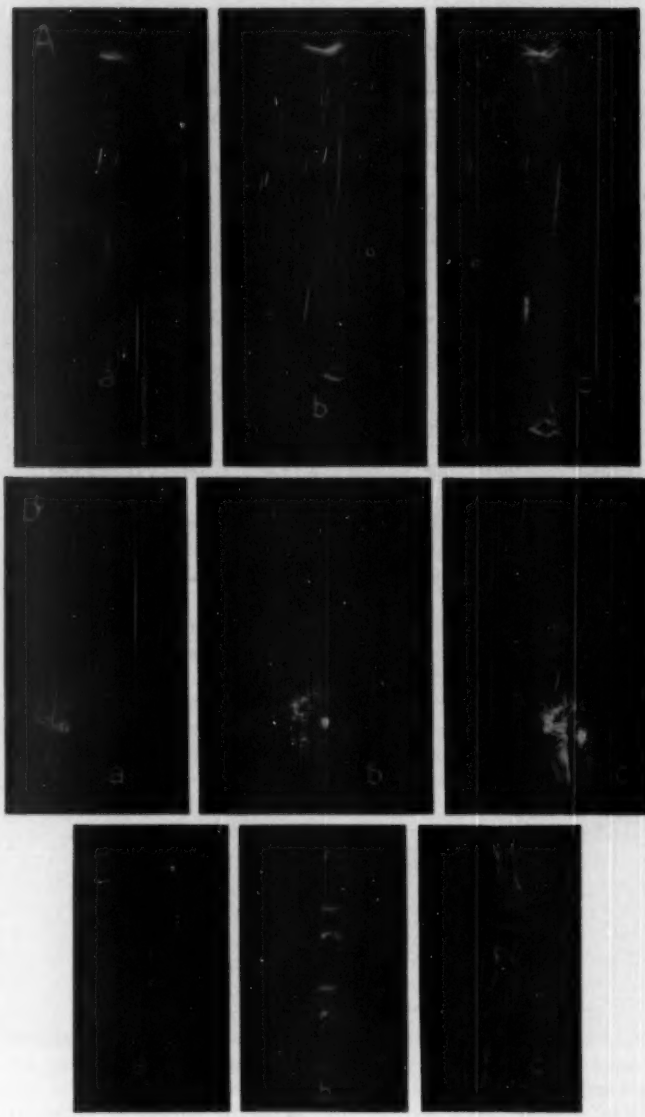

Fig. 2.—Roentgenograms of rat bones (twice natural size). The rats had been fed the experimental and control diets, respectively, for forty-nine days, and all were 70 days of age at autopsy. *A*, tibias: (*a*) phosphorus-deficient rat; (*b*) pair-fed control; (*c*) ad libitum-fed control.

B, metacarpal bones: (*a*) phosphorus-deficient rat; (*b*) pair-fed control; (*c*) ad libitum-fed control.

C, caudal vertebrae: (*a*) phosphorus-deficient rat; (*b*) pair-fed control; (*c*) ad libitum-fed control. The arrow indicates the ninth caudal vertebra.

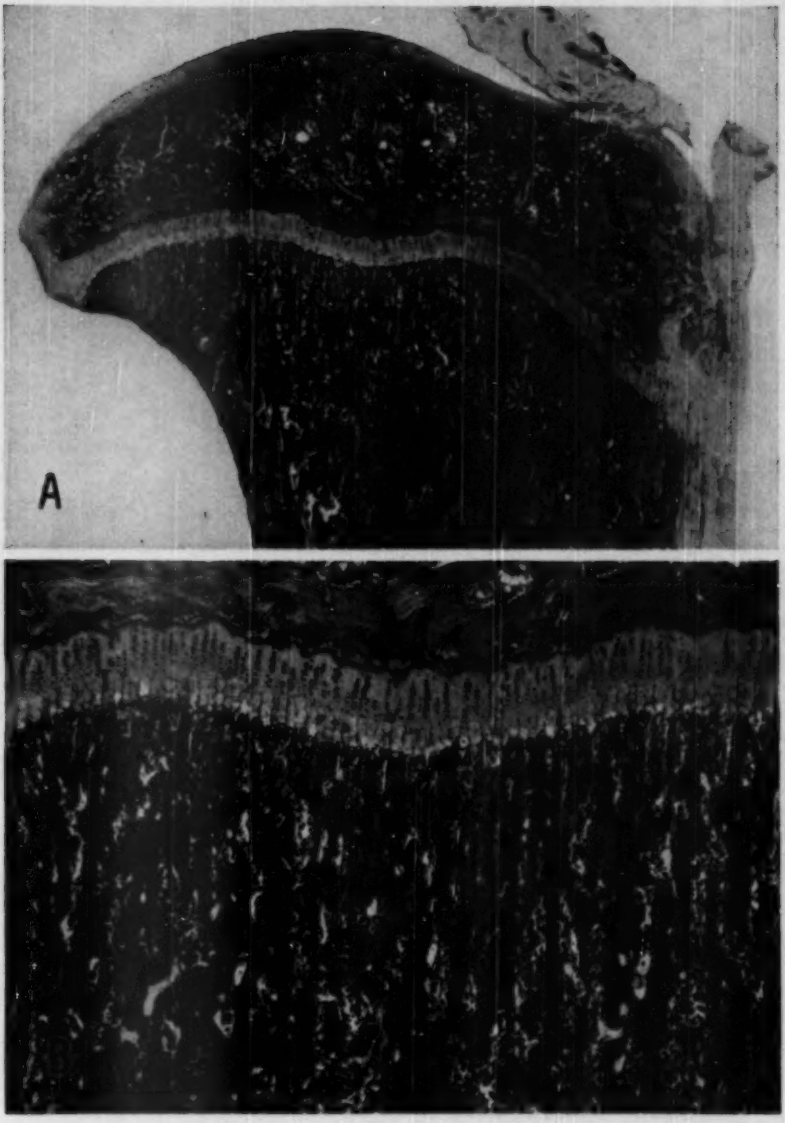

Fig. 3.—Proximal tibial epiphysial cartilage of an ad libitum-fed control female rat, 70 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 20.5. *B*, × 56.5.

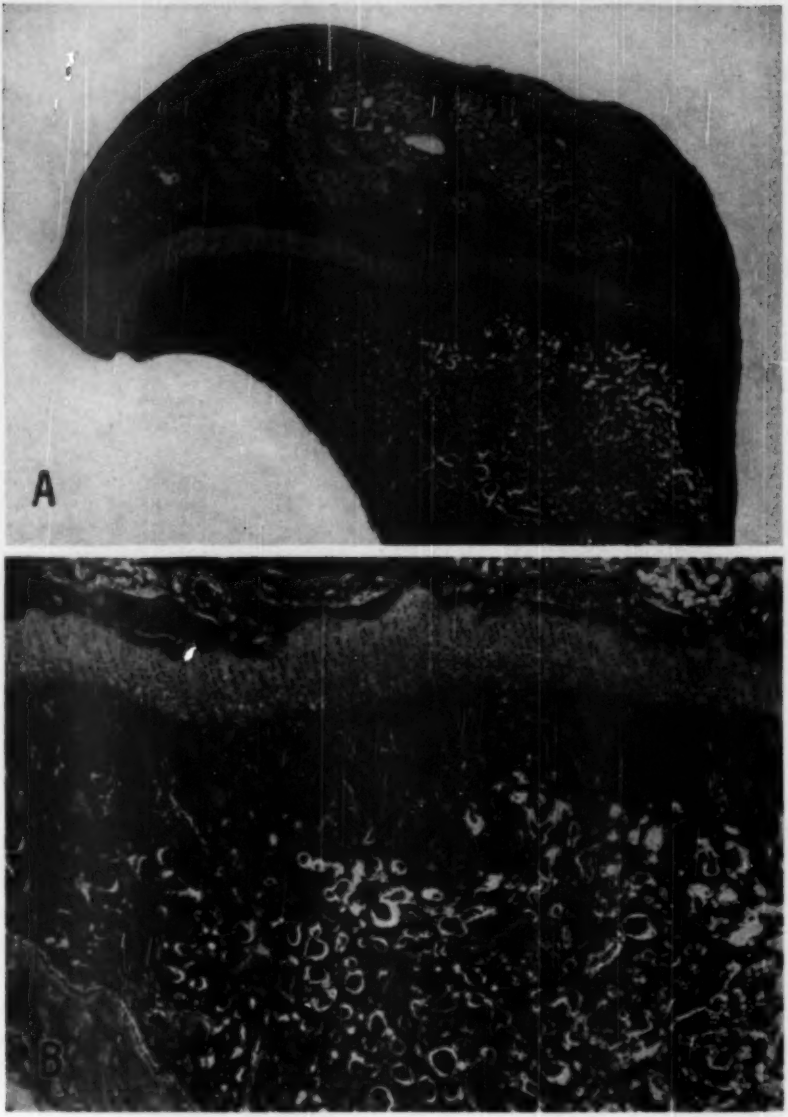

Fig. 4.—Proximal tibial epiphysial cartilage of a pair-fed normal control female rat, 62 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 25. *B*, × 68.

delicate and parallel trabeculae consisting almost entirely of lamellar bone are observed in the primary spongiosa. Many osteoblasts indicative of very active osteogenesis are seen on the surfaces of the trabeculae. Only a small amount of adipose tissue is visible. The cortical bone of the shaft appears calcified except for a narrow border of osteoid on the endosteal surface of the diaphysis.

The bones of the pair-fed control rats (fig. 4) conform to almost the same standards as the rats fed ad libitum. They were, however, slightly smaller in size, and the histologic variations may be attributed to the limitation of the food intake. In all bones the width of the epiphysial cartilage was slightly less on the average as compared with that of the ad libitum rats.

TABLE 4.—*Comparison of the Average Skeletal Measurements*

Group	Treatment	Ca:P Ratio of Food	Phosphorus-Deficient Diet, Days	Age at Autopsy, Days	Average Length			Average Width of Epiphysial Cartilage			
					Tibia, Mm.	Metacarpal, Mm.	10th Caudal Vertebra, Mm.	Tibia, Microns	3rd Metacarpal Bone, Microns	10th Caudal Vertebra	
										Mesial, Microns	Distal, Microns
1	Phosphorus-deficient	36:1	24	45	25.6	6.1	5.9	558	190	283	286
	Pair-fed	0.98:1	24	45	28.6	6.9	5.8	280	182	174	165
2	Phosphorus-deficient	36:1	32	55	25.2	5.8	4.9	554	191	278	267
	Pair-fed	0.90:1	32	55	29.3	6.7	6.1	280	127	176	165
	Ad libitum	0.90:1	32	53	30.6	6.9	6.5	240	174	186	166
3	Phosphorus-deficient	78:1	41	62	26.8	6.1	5.0	502	186	294	300
	Pair-fed	0.86:1	41	62	29.0	6.9	5.7	185	132	175	173
4	Phosphorus-deficient	31:1	69	70	24.9	5.8	4.5	370	182	266	298
	Pair-fed	0.86:1	69	70	29.7	6.9	5.9	149	107	158	152
	Ad libitum	0.86:1	69	70	33.1	6.8	7.1	186	110	169	234

Histologically, the decrease in the width of the epiphysial cartilage is principally the result of a reduction of the size of the cells and the number of cell layers in the basophilic and vesicular zones. The intercartilaginous matrix is increased in the basophilic zone. The cells of the vesicular zone have a polygonal shape, indicating the approaching end of the growth base. Osteogenesis is reduced, and there is a decrease in the number of capillaries at the line of erosion. The trabeculae adjacent to the epiphysial cartilage are short and coarse, and lateral anastomoses are prevalent. The cortical bone of the shaft is calcified except for a narrow preosseous layer on the endosteal surface.

The tibial epiphysial cartilage of the phosphorus-deficient rats (figs. 5 to 8) is in all instances markedly wider than that of the pair-fed and ad libitum–fed rats of the same age. It is not of uniform width but broad in the center and narrow toward the periphery. Abundant osteoid tissue is seen. Since there were significant differences in the

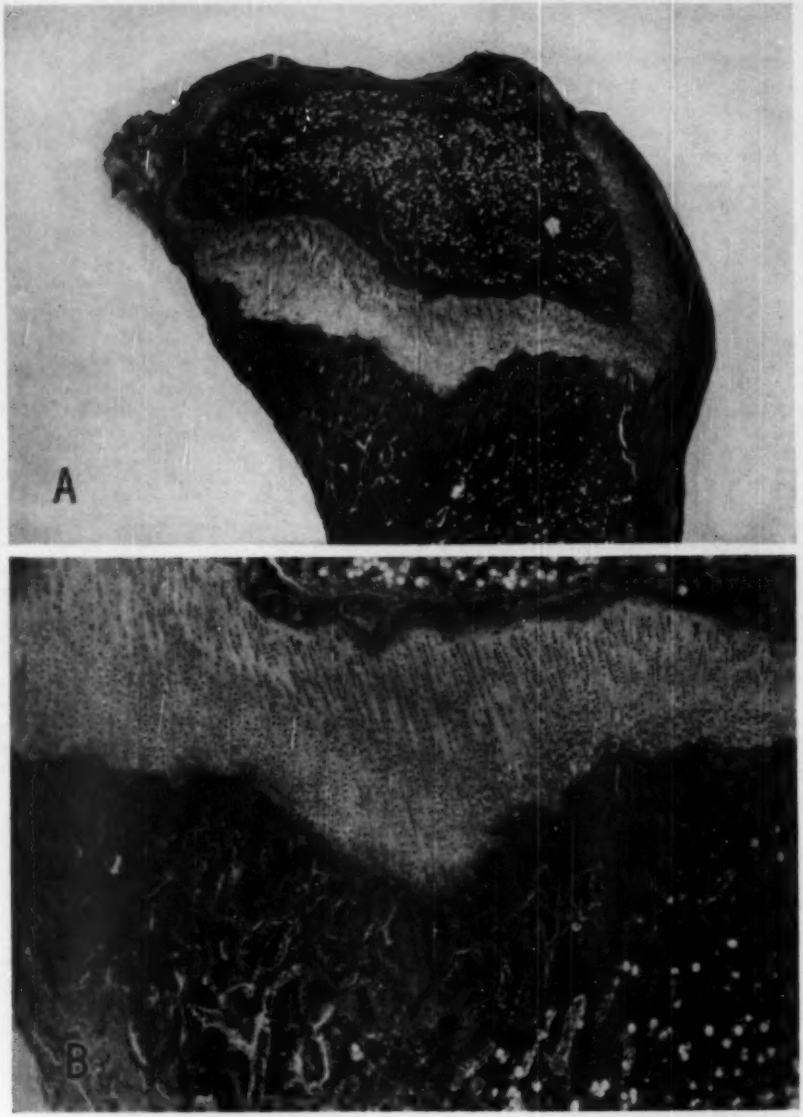

Fig. 5.—Proximal tibial epiphysial cartilage of a phosphorus-deficient female rat, which had been fed the experimental diet for twenty-four days and was 45 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 20.5. *B*, × 56.5.

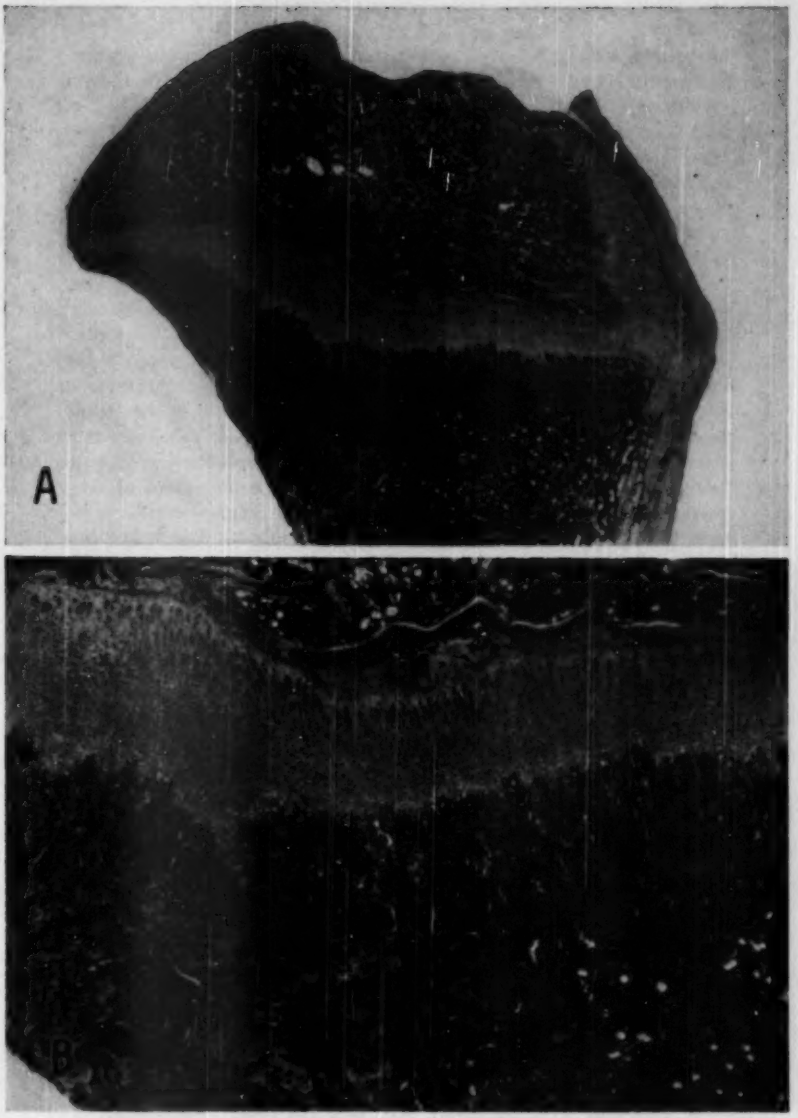

Fig. 6.—Proximal tibial epiphysial cartilage of a phosphorus-deficient female rat, which had been fed the experimental diet for forty-one days and was 62 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 20.5. *B*, × 56.5.

histogenesis of endochondral ossification between the first two groups and groups 3 and 4, only the tibias of groups 1, 2 and 4 are illustrated.

Figure 5 illustrates the irregular proximal epiphysial cartilage of the tibia of a phosphorus-deficient rat of group 1 which had been fed the experimental diet for twenty-four days. The zone of enlarged cells is extremely wide and accounts for the greatest increase in width of the entire cartilage. This zone was broad because of failure of the capillaries to invade the uncalcified cartilage. Although the width of the zone of the enlarged cells has increased, the individual cells have decreased in their vertical diameter, becoming shorter and flatter. The intercartilaginous matrix, as well as cartilage cells on which osteoid has been deposited, act as a barrier to further resorption of cartilage cells. These observations coincide with those of Dodds and Cameron.[14] In some cases the cartilage cells seem to have been infiltrated with osteoid after the opening of the lacuna. Osteoid is deposited about the chondrocytes, and at the same time the cartilage cells appear to assume the histologic appearance of the osteocytes. The failure of the cartilage to calcify and the resistance of osteoid tissue to resorption lead to this characteristic picture. There is no decrease in the number of osteoclasts even though a lack of modeling resorption is evident. The trabeculae are composed predominantly of osteoid tissue with cores of premorbid bone. Polygonal osteoblasts line the endosteal surface of the shaft, and a wide layer of osteoid matrix is formed. Similar observations have been recorded by Weinmann and Sicher[15] and Dodds.[16]

Figure 6 demonstrates the epiphysial region of the tibia after an experimental period of forty-one days (group 3). This group was fed the diet with the lowest phosphorus content and the most extreme calcium-phosphorus ratio (78:1). The epiphysial cartilage has decreased in width. It is being actively invaded by capillaries even though there is no provisional zone of calcification. Osteoid tissue is seen on both the endosteal and the periosteal surface. It appears to be deposited to a lesser degree on the periosteal surface. A dark basophilic zone extends longitudinally through the center of the cortex of the shaft. This zone is commonly recognized as normally mineralized bone. Numerous osteoblasts are visible on the endosteal surface of the shaft.

The rats of group 4 were killed at 70 days of age, after they had been on the phosphorus-deficient diet for a period of forty-nine days. This was the longest experimental period studied. The head of the tibia (figs. 7 and 8) is club shaped. The width of the epiphysial car-

14. Dodds, G. S., and Cameron, H. C.: Am. J. Path. **15**:723, 1939.

15. Weinmann, J. P., and Sicher, H.: Bone and Bones: Fundamentals of Bone Biology, St. Louis, C. V. Mosby Company, 1947, p. 261.

16. Dodds, G. S.: Am. J. Anat. **50**:97, 1932.

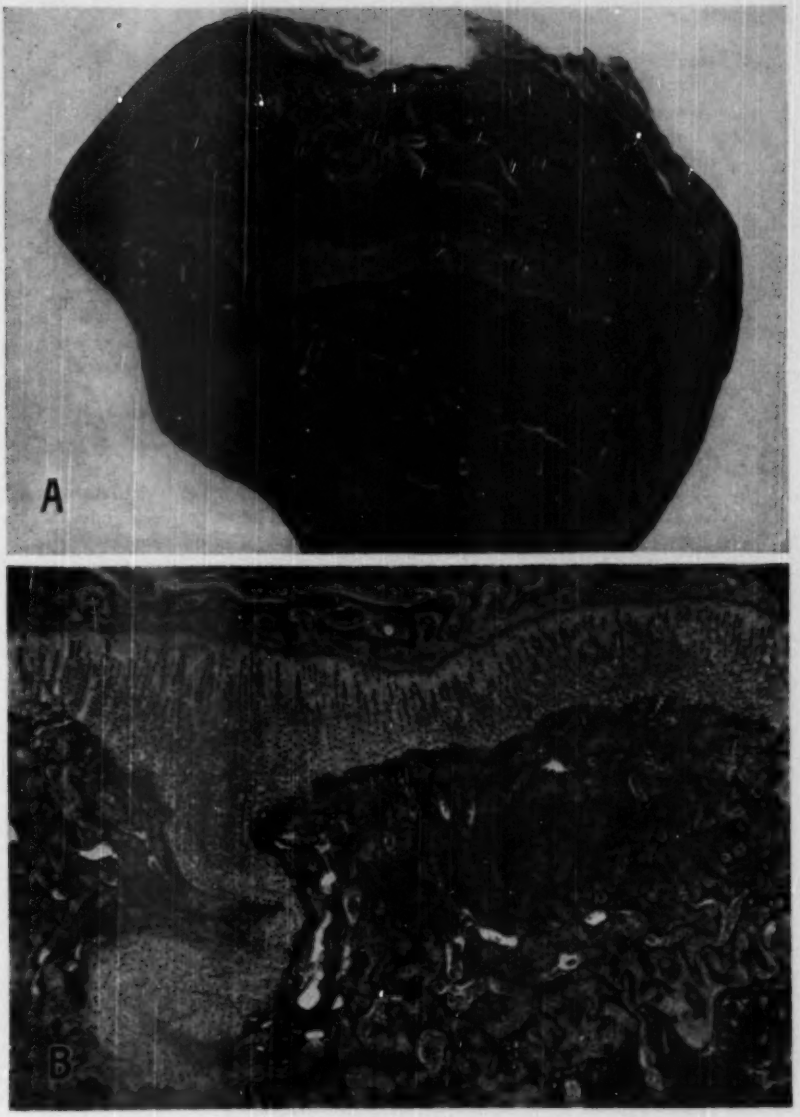

Fig. 7.—Proximal tibial epiphysial cartilage of a phosphorus-deficient female rat, which had been fed the experimental diet for forty-nine days and was 70 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 29.5. *B*, × 56.5.

tilage has further decreased, and this appears to be the result of a resumption of the invasion of the uncalcified cartilage. The removal is more pronounced at the periphery, possibly because of the larger number of blood vessels entering the marrow cavity through Volkmann's canals beneath the epiphysial cartilage. Large numbers of capillaries penetrate into the cartilage and produce masses of diversiformed osteoid trabeculae (fig. 7 *B*). The peninsula of cartilage which extends toward the shaft represents a remnant which was left in the process of cartilage removal. This renewed removal of cartilage has been described by Dodds and Cameron [9] and Bailie and Irving.[17] The cells on the diaphysial aspect of the peninsula of cartilage are flattened, while those near the epiphysis are more cuboid. The bent rows of cartilage cells probably manifest the forces of compression (fig. 7 *B*). Numerous osteoclasts are seen both at the line of erosion and on the periosteal surface of the metaphysis. Osteoid tissue is not resorbed and osteoclasts do not appear to perform their remodeling function. Only a small core of calcified bone is visible in the shaft at this age.

Figure 8 presents photomicrographs of a tibia of a rat which survived until 82 days of age. Large brushlike capillaries are seen penetrating the epiphysial cartilage in all directions. The narrowness of the epiphysial cartilage is the result of cartilage removal which was resumed between 62 (group 3) and 70 (group 4) days of age. The resumed invasion seems to occur in the older age groups and later stages of the deficiency and confirms the observations of Dodds and Cameron [9] and others.

Metacarpal Bone.—In the pair-fed controls, at 62 days of age (fig. 9 *A*) resorption and fusion of the trabeculae of the third metacarpal bone have increased the size of the marrow cavities. The trabeculae of the metaphysis are short, thick and anastomosed, and continue in thickness to the epiphysial cartilage. The blood elements in the marrow have been replaced to a large extent by adipose tissue.

Figure 9 *B* shows the changes which have occurred in the metacarpal bones of the phosphorus-deficient rats. The cortical bone of the epiphysis is narrow. The trabeculae are more numerous, short and joined with the cortex by osteoid projections. The many osteoid trabeculae remaining in the epiphysis are indicative of the lack of resorption The epiphysial cartilage is only a little wider than that of the pair-fed controls; very probably the maximum growth of the metacarpal bone has already been achieved at 62 days of age while the tibia is still in the active growing stage. The width of the basophilic zone is increased over that in the pair-fed controls. There is no evidence of a zone of provisional calcification. In contrast to that of the tibia the zone of enlarged cells is extremely narrow. The diaphysial trabeculae are

17. Bailie, J. M., and Irving, J. T.: Brit. J. Exper. Path. **29**:539, 1948.

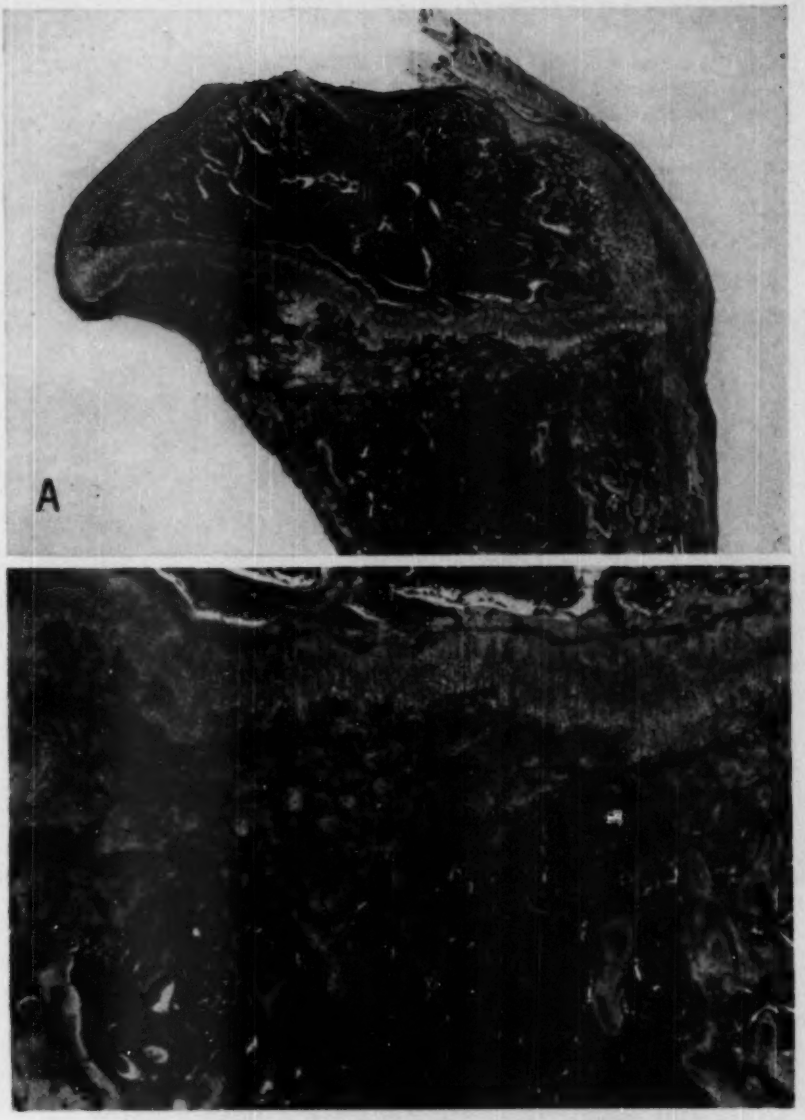

Fig. 8.—Proximal tibial epiphysial cartilage of a phosphorus-deficient female rat, which had been fed the experimental diet for sixty-one days and was 82 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 20.5. *B*, × 56.5.

thick and broad and osteoid bordered throughout. These wide osteoid layers enclose a deep staining core which presumably calcified before the experimental diet was begun.

Costochondral Junction.—The costochondral junction of an ad libitum–fed rat of group 4 is shown in figure 10*A*. Undifferentiated hyaline cartilage composed of small round cell nests is embedded in an abundant matrix. The basophilic zone lies immediately below the hyaline cartilage and consists of a zone of flattened cells arranged in columns, or several columns in the form of a fascicle. In contrast to

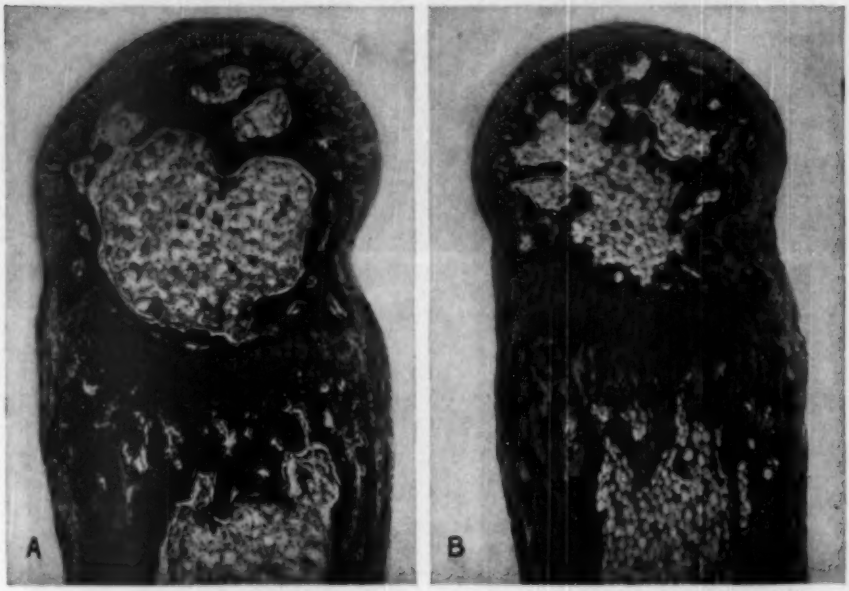

Fig. 9.—Photomicrographs of central sagittal sections of the third metacarpal bones of female rats; hematoxylin and eosin stain. *A*, pair-fed control, 62 days of age at autopsy; × 53.5. *B*, phosphorus-deficient rat, which had been restricted to the experimental diet for forty-nine days and was 70 days of age at autopsy; × 53.5.

that of the tibia the vesicular zone is narrow. The trabeculae of the primary spongiosa are numerous, long and continuous with the calcified cartilage matrix.

The costochondral junction of a pair-fed control rat of group 4 (fig. 10*B*) reveals a reduction of osteogenic activity at the line of erosion. The costochondral junction is significantly smaller than that of the ad libitum–fed control. The basophilic zone of the epiphysial

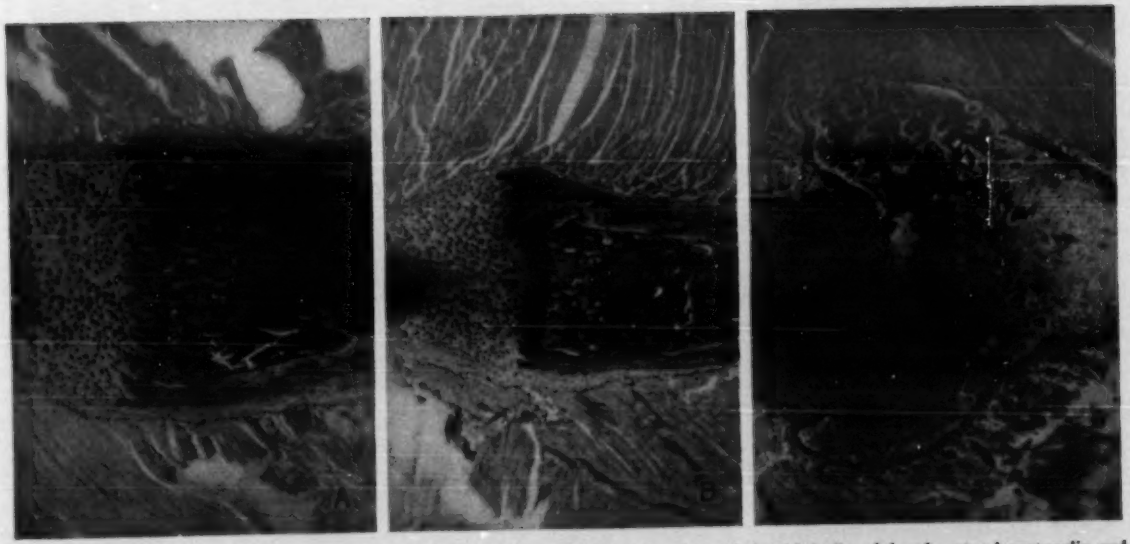

Fig. 10.—Photomicrographs of central sagittal sections of the costochondral junctions of the third ribs of female rats; hematoxylin and eosin stain. *A*, ad libitum-fed control, 70 days of age at autopsy; × 37. *B*, pair-fed control, 70 days of age at autopsy; × 37. *C*, phosphorus-deficient rat, restricted to the experimental diet for forty-nine days; 70 days of age at autopsy; × 37.

cartilage is narrow, and the cells are packed closely together. The vesicular zone is almost completely absent in contrast to the wide zone in the tibia. Only a few short trabeculae are observed adjacent to the epiphysial cartilage. These changes are associated with the restriction of food intake and limited growth of these pair-fed animals.

The costochondral junctions of the phosphorus-deficient rats of group 4 present the most severe changes in the series (fig 10 *C*). They show the classic picture of the rachitic rosary, with the length of the columns of cells in the basophilic zone greatly increased. The chondrocytes of the vesicular zone are distorted and compressed. The rib proper consists of a mass of osteoid containing islands of marrow and large capillaries at the cartilage junction. The perichondral osteoid has spread over the lateral margins of the epiphysial and hyaline cartilage, producing a bulbous effect. The normal growth in the length of the rib occurs at the costochondral junction by means of endochondral bone formation. Appositional growth occurs at the periphery, with subsequent remodeling resorption to keep the rib uniform in width. The peripheral formation of osteoid continues, and because of lack of mineralization, remodeling resorption does not take place. The surrounding tissues mold the pliable osteoid back against the hyaline cartilage. The lack of endochondral bone formation and the inability of the osteoid to be resorbed are therefore responsible for these grotesque changes.

Caudal Vertebra.—The ninth caudal vertebra of a pair-fed control rat of group 3 is illustrated in figure 11. Secondary ossification centers have formed in both the proximal and the distal end of the body of the vertebra. The marrow cavities contain considerable adipose tissue. The zone of provisional calcification is seen in figure 11 *B*. Both the proximal and the distal epiphysial cartilage are narrow at 62 days of age with an average width of 175 microns. The trabeculae of the primary spongiosa are short, coarse, and show considerable anastomosis.

The ninth caudal vertebra of the phosphorus-deficient rat (fig. 12) is considerably smaller than that of the pair-fed control. There are almost no bony trabeculae in the epiphyses. Only spicules of calcified intercartilaginous matrix with narrow osteoid borders are observed. The small secondary ossification centers have formed very recently, and this confirms the roentgenographic evidence of retarded maturation. The largest amount of osteoid tissue is on the periosteal surface of the shaft. The width of the epiphysial cartilage (fig. 12 *B*) is broader than that of the pair-fed control. The average width of proximal and distal epiphysial cartilage is 264 microns. As the cartilage cells approach the line of erosion, they become distorted and compressed. Numerous convoluted osteoid trabeculae are visible, containing blue-staining mineralized cores.

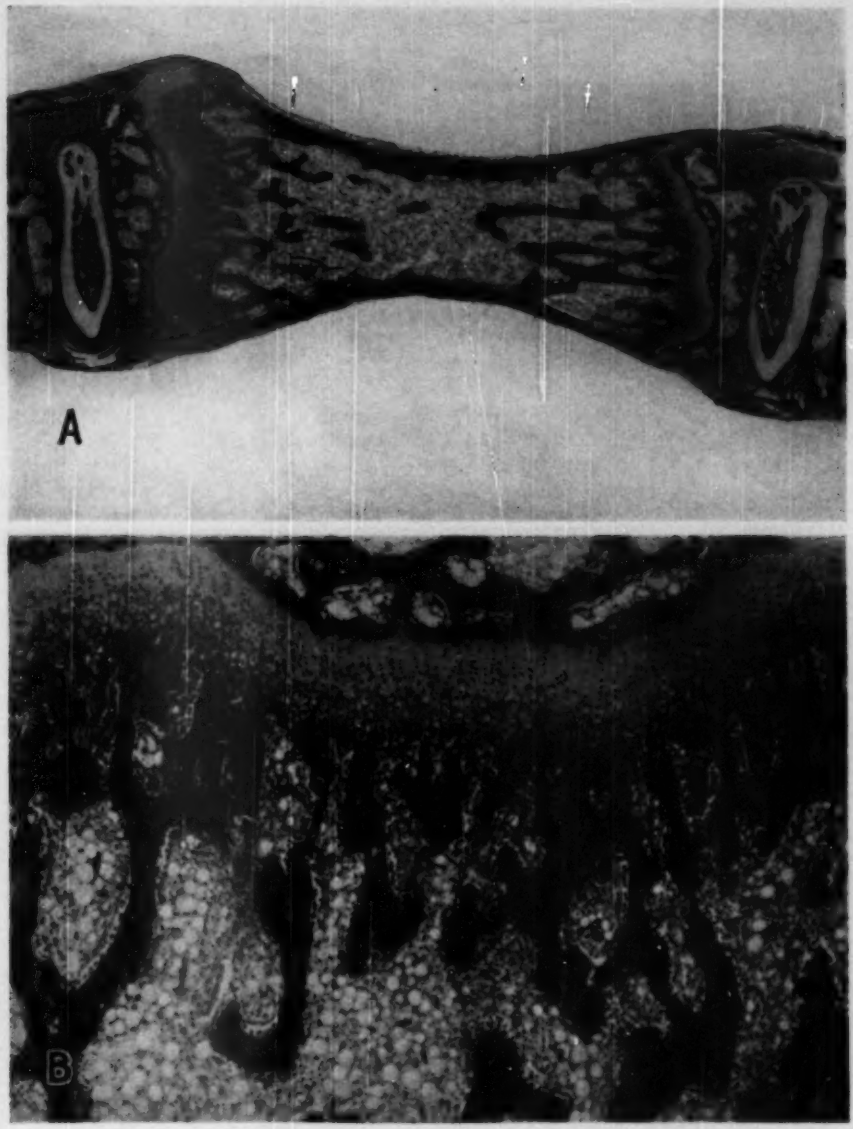

Fig. 11.—Photomicrographs of the ninth caudal vertebra of a pair-fed control female rat, which had been fed the phosphate-supplemented control diet for forty-one days and was 62 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 17.5. *B*, × 80.

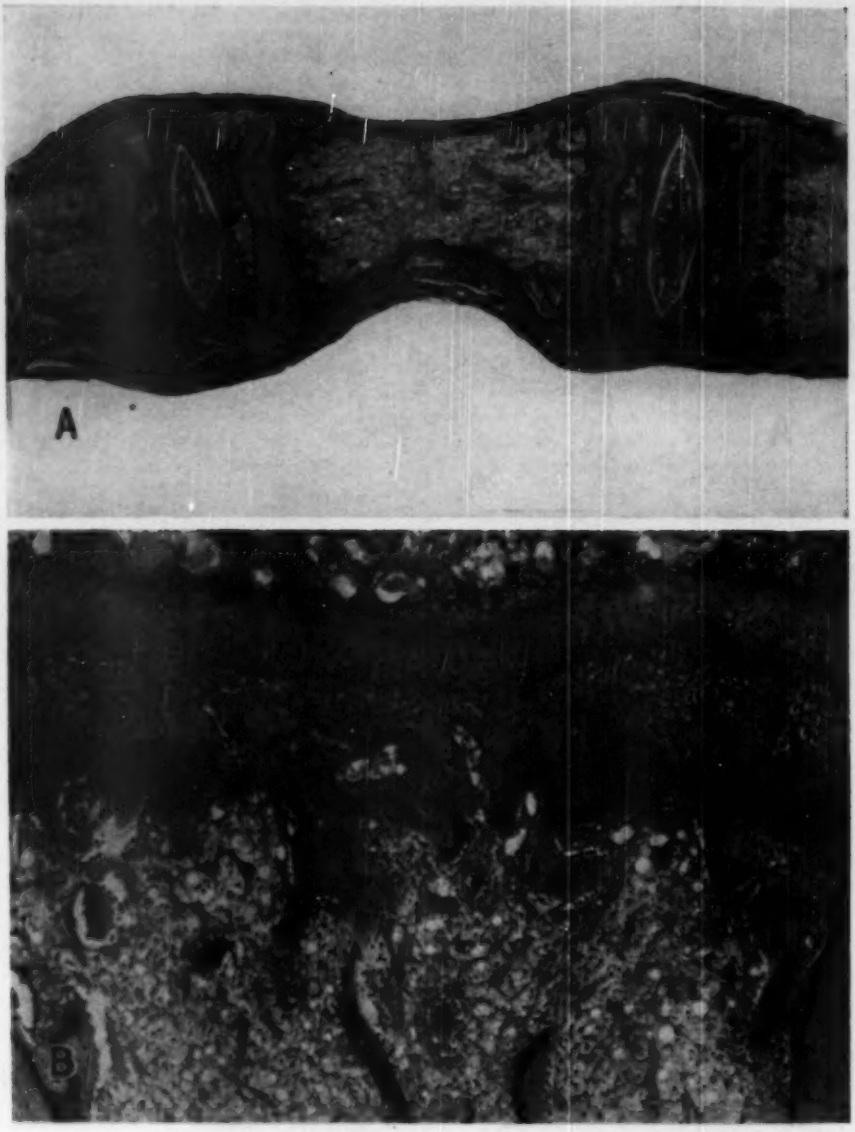

Fig. 12.—Photomicrographs of the ninth caudal vertebra of a female rat which had been restricted to the phosphorus-deficient diet for sixty-one days and was 82 days of age at autopsy; central sagittal section; hematoxylin and eosin stain. *A*, × 17.5. B, × 80.

COMMENT

The experiments described were planned to enable us to study the effects of severe phosphorus deficiency on several bones of the rat skeleton. In spite of the presence of adequate amounts of vitamins A and D, severe rickets was produced, as seen roentgenographically and histologically. It must therefore be assumed that the phosphorus deficiency was the most important factor in the production of rickets.

Furthermore, the degree of severity of the rachitic disturbance depended on the age of the animals and the length of the experimental period. Roentgenograms of the bones studied revealed a lack of mineralization and stunted growth as compared with those of the pair-fed and ad libitum–fed controls. They also showed retardation in skeletal maturation. The first pathologic change noted in all the bones was the failure of the intercartilaginous matrix of the zone of enlarged cells to calcify. This failure to mineralize may be associated with the low level of inorganic blood phosphate in the deficient animals. The capillary and connective tissue invasion of the uncalcified cartilage is not only retarded but also irregular. Multiplication of the chondrocytes was not immediately impeded, but failure of calcification and of subsequent resorption resulted in a larger number of cells, with an increase in the width of the epiphysial cartilage. The capillary invasion may have been inhibited by the lack of calcification or by the phosphorus deficiency per se. The result of these changes in cartilage was a retardation of that phase of bone growth which is dependent on endochondral ossification.

After forty-one days of the phosphorus-deficient diet there was observed a partial invasion and erosion of the uncalcified cartilage. This was evidenced by large capillaries which appeared at the line of erosion. The animals killed after forty-nine days of the diet showed these capillaries invading the uncalcified cartilage. The renewed invasion of cartilage may be related to the genetic and endocrine changes which occur in the rat at about the age of 70 days. It is known that invasion of cartilage and healing occur in rickets after a period of starvation. No evidence of calcification of the vesicular zone was detected before the removal of cartilage began. It has been shown [10a] that at approximately the same age as the resumed invasion of the cartilage manifests itself there is a slowing of the growth processes in the rat. The growth of cartilage fails to keep pace with the removal of these cells at the line of erosion, and the epiphysial cartilage becomes narrowed in width.

The osteoblastic formation of the organic matrix appears undisturbed, but the second phase, the calcification of the matrix, does not occur. The rate of formation of the bony matrix, however, does not appear to be increased. It is probably the failure of the osteoid tissue to be resorbed which prevents the remodeling of the various bones as they

grow in length. This lack of resorption would account for the gross deformities seen in the tibia and the costochondral junction of the rib. It is interesting to observe that the histologic severity of the rickets was not increased appreciably by an increase of the Ca:P ratio from 28:1 to 78:1 but did appear to increase with the age of the animals and the time during which they had been restricted to the low phosphorus diet.

Metabolic balance studies [18] revealed a negative phosphorus balance and loss of bone ash in the deficient rats. It appears that phosphorus may be removed from bone to provide for the essential phosphorus needs of the soft tissues.[7] There was a positive phosphorus balance in the case of both pair-fed and ad libitum–fed control rats, and their bones were well mineralized.

SUMMARY AND CONCLUSIONS

Severe rickets developed in growing rats restricted to diets very low in phosphorus (0.005 to 0.015 per cent) despite the presence of presumably adequate amounts of vitamins A (25 U.S.P. units per gram) and D (8 U.S.P. units per gram).

Roentgenograms showed a marked lack of mineral in the skeleton of the deficient animals, and a delay of skeletal maturation in the vertebrae.

Histologic studies were made of tibia, metacarpal bone, caudal vertebra and costochondral junction. Signs of severe rickets were evident in all four bones. The following changes were observed in the epiphysial region: (*a*) increased width of the epiphysial cartilage; (*b*) lack of a provisional zone of calcification; (*c*) failure of epiphysial cartilage to be resorbed; (*d*) compression of chondrocytes; (*e*) formation of osteoid tissue; (*f*) lack of remodeling resorption of osteoid matrix; (*g*) blood vessel invasion of the cartilage with osteoid matrix replacing it, at approximately 60 to 70 days of age.

While an increase in the Ca:P ratio from 28:1 to 78:1 did not appear to intensify the severity of rickets in these animals, definite changes were observed with increasing age and time on the low phosphorus diet.

The gross deformities of the bones in rickets may be attributed to the failure of the intercolumnar matrix and osteoid matrix to calcify. Remodeling resorption also fails to take place and the volume of osteoid is increased.

18. Kohl, F. van N.: Unpublished data.

CHANGES INDUCED IN THE CONNECTIVE TISSUE OF THE PUBIC SYMPHYSIS OF THE GUINEA PIG WITH ESTROGEN AND RELAXIN

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THE SEPARATE identity of the hormone relaxin seems at present to be well established. Allen [1] defined it as "an active substance secreted by the corpus luteum which facilitates relaxation of the pelvic ligament of certain mammals during pregnancy." The work of Hisaw [2] and of Fevold, Hisaw and Meyer [3] on relaxin as a unique hormone inducing symphysial separation in the guinea pig and other animals was confirmed by Dessau,[4] Abramowitz, Money, Talmage, Kleinholz and Hisaw [5] and by Hall and Newton.[6] Talmage [7] has described relationships between certain hormonal activities and morphologic and cytologic changes in the symphysis pubis. He showed that in mature female guinea pigs the cartilage of the symphysis pubis becomes replaced by fibrous connective tissue. During pregnancy the connective tissue proliferates in such a way that separation and relaxation of the pubes take place. These changes were thought to occur in two stages: (1) separation of the pubic bones by a preliminary conditioning provided by the estrogenic hormone, with the rapid production of fine, fibrous connective tissue; (2) relaxation, caused by actual tissue destruction of symphysial cartilage and dissolution of collagenous fibers.

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1. Allen, E.; Hisaw, F. L., and Gardner, W. U., in Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, Chap. 8, p. 453.

2. Hisaw, F. L.: Proc. Soc. Exper. Biol. & Med. **23**:661, 1925-1926.

3. Fevold, H. L.; Hisaw, F. L., and Meyer, R. K.: Proc. Soc. Exper. Biol. & Med. **27**:604, 1929-1930.

4. Dessau, F.: Acta brevia neerl. **5**:138, 1935.

5. Abramowitz, A. A.; Money, W. L.; Talmage, R. V. N.; Kleinholz, L. H., and Hisaw, F. L.: Endocrinology **34**:103, 1944.

6. Hall, K., and Newton, W. H.: Lancet **1**:54, 1946.

7. Talmage, R. V. N.: Anat. Rec. **99**:91, 1947.

The object of the present work is to attempt an explanation of the relaxation stage of symphysial separation in the light of new knowledge of connective tissue contributed by Gersh and Catchpole.[8] According to these workers, the ground substance, defined as the intercellular material surrounding cellular and fibrillar elements, is composed of a homogeneous glycoprotein of nonfibrillar nature. The ground substance is presumed to be structurally organized at a submicroscopic level, and polymerized to a degree that may vary with the physiologic state. When highly polymerized, the ground substance exhibits the properties of water insolubility, and of weak stainability (affinity) with Evans blue dye used intravitally. With the ground substance less highly polymerized, these properties are altered in the direction of greater water solubility and ready stainability with Evans blue. Granules found in fibroblasts with the same staining properties as the ground substance seemed to follow a secretory cycle and were interpreted by Gersh and Catchpole as being possibly related to the secretion of ground substance. In addition, secretion of enzymes of the mucinase class was invoked to explain the varying degrees of polymerization of the ground substance.

An explanation of the phenomenon of relaxation may lie within the bounds of these alterations occurring in the physical state of the ground substance. In adult castrated female guinea pigs, the symphysial ground substance appears to be in a state of high polymerization. In this condition the microscopic and submicroscopic elements—cells, fibers and fibrils—are thought to be bound together tightly in patterns which provide a firm connective tissue matrix. In the relaxed symphysis the ground substance has undergone a depolymerization resulting in varying degrees of separation of the intracellular elements. Grossly, then, the joint of the symphysis becomes mobile and flexible, and preparturition separation is possible. A preliminary account of changes in the symphysis bearing on this explanation has been given by Perl and Catchpole.[9]

ANIMALS AND METHODS

Symphysial tissue was obtained from young adult guinea pigs. Some animals were used intact; others were castrated and used a month after operation. Particulars and treatment of these animals may be summarized:

Group 1. Intact and castrated animals which had not been treated with estrogen or relaxin.

Group 2. Castrated animals which received 1 microgram (0.001 mg.) of estradiol benzoate[10] in 0.1 cc. of sesame oil subcutaneously for five days.

Group 3. Castrated animals which received 1 microgram of estradiol benzoate in 0.1 cc. of sesame oil daily for five days, then 1.0 cc. of pregnant rabbit serum con-

8. Gersh, I., and Catchpole, H. R.: Am. J. Anat. **85**:457, 1949.

9. Perl, E., and Catchpole, H. R.: Federation Proc. **8**:126, 1949.

10. The estradiol benzoate was obtained from the Schering Corporation, Bloomfield, N. J., through the courtesy of Dr. Norman Hemenway.

taining relaxin. They were killed six to twelve hours after the administration of relaxin.

Group 4. Pregnant animals bearing fetuses approximately 70 to 100 mm. in length. The actual age of the fetuses was unknown.

Method of Estimating Intravital Staining with Evans Blue.—After the hormonal priming described, all animals received 4.0 cc. of 1.25 per cent Evans blue solution intravenously and were killed ten minutes later with ether and pneumothorax. The symphysial regions of half the animals were removed *in toto* and immersed directly in isopentane at —160 C., in which the symphyses were rapidly frozen; in this way the pattern of Evans blue localization was preserved. With the remaining animals, symphysial tissues were removed and partially dissected before freezing in isopentane. This material was used for detailed cytologic study. All the tissues were dried in vacuum tubes at —30 C. When ready, the symphyses were infiltrated for fifteen minutes with soft paraffin (melting point 40 C.) for sections to be examined for Evans blue, or in harder paraffin (melting point, 56-58 C.) for cytologic investigation, and finally embedded. Thick sections (120 microns) were made for the clearer visualization of Evans blue. They were mounted on albuminized slides with slight pressure and warmth. Paraffin was removed from the slides with several changes of heated liquid petrolatum, and the sections were covered with a cover slip. Intravital staining with Evans blue was used to determine whether in the symphyses, as in other sites, Evans blue was more concentrated in areas where the ground substance had become water soluble.

Method for Determining the Solubility of Ground Substance.—Thinner sections (20 microns) were stained by the periodic acid–leukofuchsin method.[11] The stain is given by polysaccharides and by other sugar-containing substances and involves the oxidation of contiguous hydroxyl groups of sugar residues yielding aldehyde groups which are held in high molecular combinations. These give compounds with the fuchsin sulfite reagent of varying shades of pink and red when viewed microscopically. By suitable control methods, the technic may be made specific for glycoproteins.[8] The color density in the sections is an indication of the number and the compactness of the reactive groups and provides a first indication of the nature of the ground substance. By comparing two adjacent sections treated differently but stained simultaneously, it is possible to estimate whether, and to what degree, the reactive substance is water soluble.

For the initial control studies, a few sections from each symphysis were denatured in absolute alcohol for twenty-four hours, then stained for glycoprotein with periodic acid–leukofuchsin. Other sections from the same tissues were treated with a few drops of phosphate buffer at p_H 7.0 and incubated for two hours in order to test the solubility of the ground substance. After extraction, the sections were denatured in absolute alcohol and stained as before. Any decrement in staining then indicates the removal of water-soluble glycoprotein material.

RESULTS

After the guinea pigs had been treated with the estrogen, relaxin or both, they were classified into (1) animals in which the preliminary estradiol treatment was not sufficient to result in relaxation of the

11. McManus, J. F. A.: Nature, London **158**:202, 1946. Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

symphysis and (2) animals which had received estradiol and relaxin in sufficient doses to cause relaxation of the symphysis pubis. Findings in the first group were in most respects similar to those in the untreated controls.

In controls and in treated animals which did not show relaxation the ground substance of the symphysis appeared to be in a polymerized state. With the McManus-Hotchkiss method, the connective tissue was pale pink in the control sections, and this appearance was unchanged in the extracted and denatured sections. Glycoprotein granules were absent from the fibroblasts, and the basement membrane of the blood vessels was sharply demarcated (table 1). Evans blue was found only in the blood vessels of the region, with occasional traces in the wedge-shaped con-

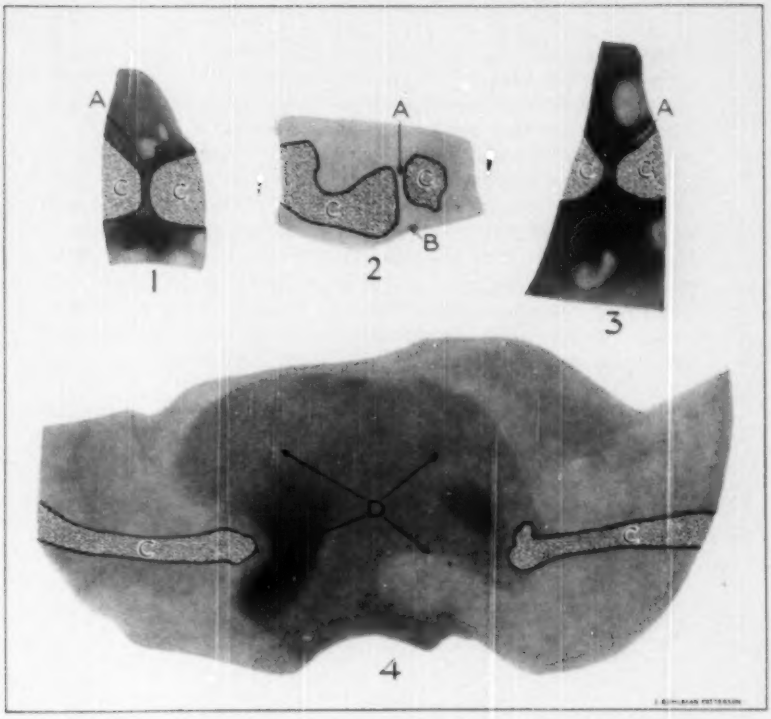
TABLE 1.—*Localization and Staining Intensity of Glycoprotein-Containing Materials in the Pubic Symphyses of Guinea Pigs Before and After Treatment with Estradiol and Relaxin, and in Late Pregnancy*

Group	Animals	Treatment	Depth of Staining of the Ground Substance of Connective Tissue*		Basement Membrane of Blood Vessels of Symphysis	Granules in Fibroblasts
			Before Buffer	After Buffer Extraction at pH 7.0		
1	4	2 normal 2 castrated for 1 mo.	Pale pink	Pale pink	Present	None
2	3	Castrated, treated with estradiol	Pink	Pink	Present	None
3	4	Castrated, treated with estradiol and relaxin	Pink to red	Paler	Absent	Present in irregular amounts
4	3	Pregnant	Pink to red	Paler to much paler	Absent	Numerous or absent

* The periodic acid-leukofuchsin method of McManus and of Hotchkiss was used. This gives a red or pink color with glycoproteins.

nective tissue areas lying adjacent to the bone, cartilage and connective tissue on the anterior and posterior borders of the symphysis (*2* in figure). Estradiol-treated animals showed a distinct increase in dye deposition as compared with controls (*1* in figure). The dye tended to be fainter in the connective tissue of the immediate intersymphysial space.

In contrast with the findings in the unrelaxed symphyses, the ground substance in the pubic regions of guinea pigs which had been treated with relaxin or which were in advanced stages of pregnancy appeared to be in a depolymerized state (table 1). With the McManus-Hotchkiss method, the connective tissue of the denatured sections stained more deeply, and the fibrillar elements revealed looser, less compact networks than were seen in the control symphyses. In the blood vessels in these sections the basement membrane was poorly differentiated. The extracted



EXPLANATION OF FIGURE

The figure illustrates the manner in which Evans blue was deposited in the pubic symphyses of guinea pigs. These semidiagrammatic drawings were made with the camera lucida from the cut surfaces of blocks of tissue which had been frozen, dried and embedded in paraffin. As distinct from sections of these blocks, a considerable depth of color is now visible.

Each animal received an injection of 4.0 cc. of 1.25 per cent Evans blue and was killed by bleeding ten minutes later. A large block of tissue was removed to minimize mechanical shifts of dye, frozen in isopentane and dried *in vacuo*. The dried block was trimmed and then embedded in paraffin.

Key: *A* indicates the intersymphysial region; *B*, anterior and posterior connective tissue wedges; *C*, the pubic bones; *D*, the symphysial area of bone resorption of pregnancy.

1, castrate female treated with 1 microgram (0.001 mg.) of estradiol benzoate in oil daily for five days. Dye is present in the intersymphysial region and to a greater extent in the anterior and posterior connective tissue wedges. × 7.75.

2, castrate female, control. There is faint color in a wedge area; otherwise dye is absent. × 7.75.

3, castrate female treated with 1 microgram of estradiol benzoate in oil daily oil daily for four days, then given 1 cc. of pregnant rabbit serum containing relaxin, treated with Evans blue and killed six hours later. Dye appears in all regions of the symphysis, and particularly the wedge areas are densely stained. × 7.75.

4, pregnant guinea pig in late stage, bearing 2 fetuses of length 98 and 102 mm. There is a gap of about 1 cm. between the pubic bones. Dye is present in the intersymphysial region and appears diffusely and widely in the wedge areas. × 5.25.

sections were generally paler and in many cases appeared partially dissolved. In both denatured and extracted sections there were glycoprotein granules in the fibroblasts, which stained with the same intensity as the ground substance. Sections from the relaxin-treated animals always revealed numerous granules. However, in a number of sections from pregnant animals fibroblasts of the symphysis were seen to be completely devoid of intracellular granules.

Evans blue dye was found in greatest concentration in those areas of the symphyses of relaxin-treated animals which showed changes in polymerization. Blue was seen in the actual symphysial connective tissue (table 2). Under subdued light the dye was generally apparent as anastomosing streaks in the most vascularized areas. Often streaks of color were noted surrounding blood vessels, as if in process of diffusion.

TABLE 2.—*Localization of Evans Blue in Blood Vessels and Tissues of the Symphysis Pubis of Castrate Guinea Pigs Before and After Treatment with Estradiol and Relaxin and in Late Pregnancy*

			Evans Blue Distribution*		
Group	Animals	Treatment	In Plasma of Blood Vessels	In Ground Substance of Connective Tissue Between Cartilage	In Ground Substance of Connective Tissue of Wedge Areas
1	2	Control	+++	0	Trace or absent
2	3	Estradiol	+++	0	Trace or absent
3	6	Estradiol-relaxin	+++	Blue	Deep blue
4	6	Pregnant	+++	Blue	Deep blue

* The symphysis was fixed *in toto* in isopentane at —160 C., dehydrated *in vacuo* and embedded in paraffin. Sections were made at 120 microns. For visualization of dye in whole block mounts, see figure.

Occasionally the blue areas overlapped, producing a less sharply defined blue region of greater color intensity. In all instances the color of the connective tissue of the wedge area was markedly darker than that of the symphysis in the same section (*3* in figure); in some animals the color was seen to extend even into adjacent muscular bands for a small distance. Most of these appearances were present to a striking degree in animals in late stages of pregnancy (*4* in figure).

It is suggested that the Evans blue localization which is found in "primed" guinea pigs after administration of relaxin may provide a more objective assay technic than the present method of palpation.

COMMENT

The changes observed in the intersymphysial connective tissue of guinea pigs are believed to be the result of depolymerization of ground substance. This, stated in physical terms, involves the breaking down

of complex organized glycoprotein molecules into a greater number of highly reactive subgroups. In the presence of periodic acid–leukofuchsin dye these subgroups react more completely and hence appear more deeply colored than do the compact elements of a polymerized tissue. The breakdown may be so extensive that groups previously water insoluble are converted into molecules readily dissolved in a buffer solution at p_H 7. Sections thus treated with buffer were less deeply stained.

It is possible that the sporadic presence of the glycoprotein granules in the fibroblasts may be explained in terms of a secretory cycle related to deposition of ground substance. If this is the explanation, the cycle can be reviewed as having four phases: (1) resting phase, (2) phase showing beginning secretion, (3) phase showing peak of secretion and (4) phase showing exhaustion. The lack of granules in the symphyses of castrated control animals could represent a resting stage where the granules are scarce or absent, in accord with the presumed slow rate of ground substance replacement in this state. The sections revealing occasional dispersion of granules can be considered as showing the beginnings of a secretory function. Symphysial sections from the pregnant animals might represent both the third and fourth phases, since the fibroblasts were flooded with granules in some instances and devoid of granules in others. This may indicate that granules reach a peak of secretion, after which they disappear from sight to remain inactive in the "resting state" until subsequently a new cycle begins.

The localizing of Evans blue in the symphysis has been used as an indication of the degree of depolymerization of the ground substance. Ordinarily the dye does not pass through vascular membranes to any considerable extent, but in relaxed animals Evans blue was found in varying quantities in the connective tissue of the symphyses and of surrounding regions, and the basement membrane of the blood vessels in this area was seen to be affected in the same manner as the symphysial ground substance. This membrane appears to undergo a process of thinning or dissociation that is possibly related to its greater permeability for such plasma components as the intravital dye. In addition, it has been suggested that active groups resulting from the depolymerization of the ground substance might combine chemically with the dye.[8]

The deep coloration of the wedge areas and the streaks of blue seen running into the connective tissue of the bordering muscle most probably imply that depolymerization is not limited simply to the ground substance of the symphysis pubis proper, but may extend to the ground substance in other areas related to the physiologic functions of relaxation. Thus, Pommerenke[12] observed that mobility of the pelvic joint was

12. Pommerenke, W. T.: Am. J. Obst. & Gynec. **27**:708, 1934.

increased during pregnancy by relaxation of the interpubic ligament and of the sacroiliac articulation. Gardner[13] found that bilateral scrotal hernias generally developed in male mice receiving estrogens owing to relaxation of the scrotal ligament accompanied with some changes in the pelvic bones. Recent investigations have shown even more startling associations in arthritis.[14] Arthritic joints of women were reported to have become mobile and in many cases completely cured when the patients became pregnant. Perhaps, also, more distantly related phenomena illustrated by the stretch of the abdominal wall in late pregnancy and its return to normal after parturition may be concerned with active depolymerization of the ground substance of muscle.

On the basis of the evidence given in the foregoing pages it is possible to attempt a physicochemical explanation of the phenomenon of preparturition relaxation. After having been stimulated with the hormone relaxin subsequent to a conditioning with estrogens, the symphysial ground substance of the guinea pig undergoes changes which are believed to be depolymerizing in nature. The ground substance which is thought to exist normally in a state of high intermolecular organization, becomes more loosely arranged; the tissue assumes a more fluid consistency and a degree of mobility is attained.

While the behavior of individual fibers was not studied in this work, if the concept is valid that fibers and fibrils are secured in a matrix of ground substance, a weakening of the latter will lead to disorientation of fibers, irrespective of intrinsic changes in the fibers themselves. Concomitant changes in fibroblasts of the region, namely, increase in size and granularity, and frequently a picture of exhaustion of granules, suggest a cycle during which the ground substance is being more or less rapidly synthesized.

SUMMARY AND CONCLUSION

After estrogen-relaxin treatment the ground substance of the symphysis pubis of the guinea pig becomes more deeply stainable with periodic acid-leukofuchsin and more water soluble. Intravenously administered Evans blue becomes localized in these regions.

The mechanism of preparturition relaxation is interpreted in terms of depolymerization of the ground substance of the connective tissue of the symphysis pubis and of contiguous regions.

13. Gardner, W. U.: Am. J. Anat. **59**:459, 1936.
14. Hench, P. S.: Proc. Staff. Meet., Mayo Clin. **13**:161, 1938.

ARTERITIS OF THE APPENDIX

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NECROTIZING arteritis of appendical arteries may produce signs and symptoms suggestive of acute appendicitis. The condition usually occurs in an otherwise normal organ or in one that reveals either a mild chronic inflammatory reaction or is partially obliterated. Rarely is there an associated acute appendicitis. The arteritis simulates in appearance the change present in the small arteries and arterioles in polyarteritis nodosa so closely as to cause one to consider the possibility that the lesion represents a local manifestation of this disease. The changes are also similar to those occurring in the small peripheral arterioles and capillaries, either systemic or pulmonary, in rheumatic cardiac disease,[1] and to those present in the arterioles of the kidney in "malignant hypertension." In respect to the latter, Fahr [2] expressed the opinion that a sharp distinction cannot be drawn between the necrotizing arteriolitis of the kidney in "malignant nephrosclerosis or hypertension" and the extrarenal lesions of polyarteritis nodosa. It does not resemble the arteritis produced by the acute inflammatory reaction of acute appendicitis.

The first description of this arterial change was given by Plaut,[3] who stated:

> . . . the lesions have been found in many routine specimens of the appendix vermiformis and occasionally in specimens of the internal female genital organs. They may be designated as focal nonspecific arteriolitis. I have never seen them in veins or in any medium-sized or larger arteries thus far. This focal disease occurs in young people as well as in older people. It is found in inflammed appendixes and in so-called normal appendixes taken out in the course of abdominal operations. It is located in the muscle coat and in the serosa of the appendix.

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1. von Glahn, W. C., and Pappenheimer, A. M.: Am. J. Path. **2**:235-249, 1926.

2. Fahr, T., cited by Gruber, G. B.: Virchows Arch. f. path. Anat. **258**:441-501, 1925.

3. Plaut, A., abstracted in Am. J. Path. **8**:620-621, 1932.

Scholl [4] and Fingerland [5] have reported single examples of arteritis of the appendix. Because of the paucity of reports pertaining to this lesion a study was made of its clinical and pathologic aspects.

MATERIAL

All appendixes removed at operation between Jan. 1, 1940 and June 30, 1948 were examined for arteritis. The group comprised 2,462 appendixes, in 1,345 of which the condition was diagnosed as acute or subacute appendicitis with or without perforation. The diagnoses most frequently made on the remaining 1,117 appendixes were chronic appendicitis, obliteration of the appendix, complete or partial; fecalith in the appendix, diverticulum of the appendix and nonspecific granuloma of the appendix.

Arteritis of the appendical vessels was observed in 22 of these appendixes. However, in only 1 instance was the lesion present in an appendix that also revealed evidences of acute appendicitis.

Of the 22 patients, 19 were females and 3 were males. Also of interest are the ages of these patients; 17 were young women between the ages of 15 and 23, 12 of the 17 being between 15 and 19 years of age; 1 was a 6 year old boy, 1 a 27 year old woman, 1 a 46 year old man, and 1 a 55 year old man.

PATHOLOGIC CHANGES

The gross description of the appendixes was obtained from the operative notes, since all the specimens were received fixed in either formaldehyde solution U. S. P. diluted 1:10 or in Bouin's solution. Eight were described at the time of operation as being normal in appearance. The changes observed in the others were mild in degree and consisted of injection of the serosa, a slightly bulbous tip, rarely the presence of a few adhesions and, in 2 appendixes, fecaliths.

In addition, small cysts were reported as being present in the ovaries of 4 patients.

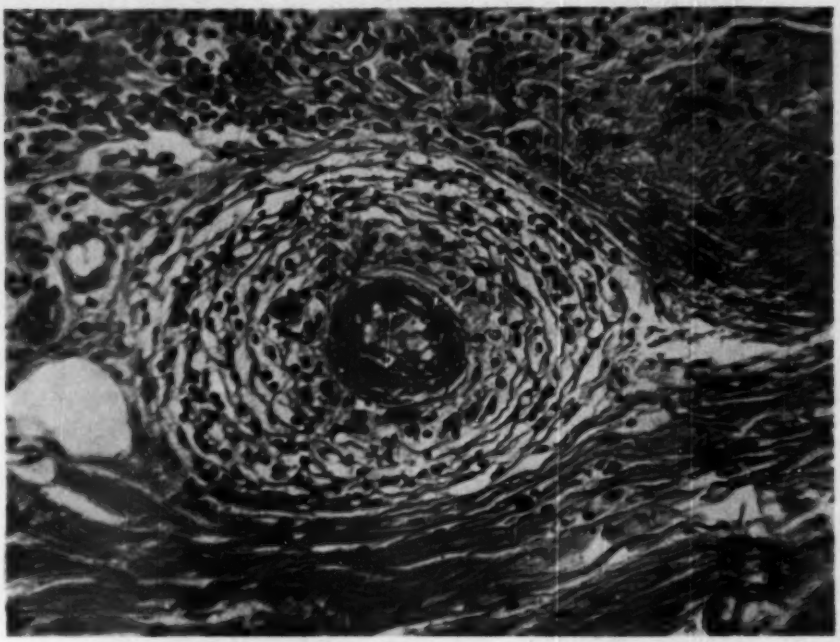
HISTOLOGIC DESCRIPTION

The histologic descriptions are based on the examination of 3 to 6 blocks of tissue obtained from each appendix. Routinely blocks of tissue for sectioning were cut from the distal, middle and proximal portions of the appendix. From these blocks 3 to 24 sections were prepared. Routinely the tissues were stained with hematoxylin and eosin.

In the vessels that revealed the arteritis the endothelial cells were swollen. Within the intima and the media, involving the entire circumference of the vessel, there was a distinct zone of homogeneous pink-staining material that in some areas had the appearance of necrotic tissue while in others it resembled very compact fibrin. Some of the arteries revealed nuclear debris within the necrotic media, and rarely

4. Scholl, R.: Zentralbl. f. Chir. **63**:2113-2116, 1936.
5. Fingerland, A.: Casop. lék. česk. **78**:154-156, 1939.

this process was striking both in the zone of necrosis and in the adventitial tissue. The adventitial cellular reaction consisted of a mild to moderate collection of lymphocytes, large mononuclear cells and a few polymorphonuclear leukocytes. Only one or two eosinophilic cells were observed in an occasional vascular lesion. The proportion of various types of cells varies from vessel to vessel; in some the lymphocytes were most numerous, while in others the predominant



Arteritis involving a submucosal vessel of the appendix; × 342.

cells were the large mononuclears. There was a moderate circumferential proliferation of adventitial fibroblastic tissue (figure). Sections that contained arteries cut longitudinally disclosed that only a segment of the vessel was involved in the inflammatory process.

The ultimate fate of the arteries was difficult to determine. A rare section that contained active lesions also revealed an occasional scar that could possibly represent vessels that previously were the site of an active process.

Phosphotungstic acid–hemotoxylin stain demonstrated that only a portion of the homogeneous pink-staining material of the intima and media is fibrin. The Weigert-Van Gieson stain for elastic tissue revealed the elastica interna to be thickened and fragmented in some vessels and in others completely destroyed. The area of necrosis of some of the vessels appeared to be almost entirely within the media, while in others both the intima and the media revealed this change. Bacteria could not be demonstrated in the vessel wall with either Brown-Brenn [5a] or Ziehl-Neelsen stains.

The number of arteries in an individual appendix revealing arteritis varied from 1 to 7, with an average of approximately 3. The lesions were most frequently encountered in the submucosal arterioles and arteries, being observed in this location in 17 of the 22 appendixes. There were 7 appendixes in which the lesions were present in the muscularis, and in 6 of the 22 appendixes the serosal vessels were also involved. The vessels of all three layers were involved in only 2 appendixes. This change was not noted in the arteries of the mesoappendix.

In the majority of the appendixes no lesion other than the arteritis could be found. Occasionally an additional diagnosis, either of chronic appendicitis or of partial obliteration was made. In only 1 instance was there also mild acute appendicitis.

CLINICAL OBSERVATIONS

With a few exceptions the clinical signs and symptoms could be considered to be those of appendicitis. All the patients had pain that localized to the right lower quadrant of the abdomen. The majority of the patients gave a history of similar episodes of abdominal pain over a period as long as four and a half years. Seventeen were nauseated or had vomiting at the time of onset of the abdominal pain; however, the majority of these were only nauseated. Tenderness was elicited on palpation of the right lower quadrant of the abdomen in most of the patients. Occasionally this sign was generalized or present also in the left lower quadrant. Tenderness in these areas was not associated with changes in the pelvic organs so far as could be ascertained at the time of operation. Half of the patients had some degree of rigidity of the musculature of the anterior abdominal wall.

The white blood cell count was elevated in 16 cases, ranging from 9,500 to 17,500 per cubic millimeter. In 1 instance the eosinophilic cells were increased to 5 per cent. Occasionally white or red blood cells, as well as some epithelial debris or cells, were present in the urine of 6 patients. In the blood of 2 patients the nonprotein nitrogen was

5a. Brown, J. H., and Brenn, L.: Bull. Johns Hopkins Hosp. 48:69-73, 1931.

normal. Blood pressure was normal in all except in 2 patients; it was 148 systolic and 110 diastolic in a 27 year old Negro man, who had advanced pulmonary tuberculosis and was also suspected of having polyarteritis nodosa, and 240 systolic and 120 diastolic in a 54 year old white woman with arterial hypertension of several years' duration. Body temperature ranged between 99.4 and 100 F. in all the patients with the exception of the man with pulmonary tuberculosis, in whom the temperature ranged between 101 and 102 F. The clinical diagnosis based on signs and symptoms was either acute appendicitis or "acute appendicitis to be excluded" in 17 instances; chronic appendicitis in 2 and gastroenteritis in 1; in the remaining 2 cases the diagnosis was undermined preoperatively.

COMMENT

While in some of the appendixes the arterial lesions were minimal in degree, the clinical signs and the symptoms of the patients were generally typical of appendicitis. It must be considered that the arteritis produced these signs and symptoms and therefore represents an additional cause of the clinical manifestations of appendicitis. In all probability some of these arterial changes have been missed in the routine examination of appendixes, and so the cause of the patient's complaints was not ascertained.

Of particular interest is the sex and age of the patients in whom the appendical arterial change was present. Of the 22 patients, 19 were females, 17 of whom were between the ages of 15 and 23. We are unable to give an adequate explanation for this age and sex distribution of the lesion.

The changes in the appendical vessel are similar in many respects to those described by von Glahn and Pappenheimer [1] at present in the peripheral arterioles in rheumatic cardiac disease; however, in these patients with appendical arteritis signs and symptoms that could be attributed to rheumatic fever were not present.

Plaut [3] surmised that some immunologic condition of the tissues may be responsible for the pathologic change in the arterioles. Scholl [4] suggested several possibilities: that it is an "oligosymptomatic" form of polyarteritis nodosa or a "part" of a low grade appendicitis that is a result of anaphylaxis, or is a result of the local manifestation of a generalized arteritis. Moschcowitz, in a review of the subject of polyarteritis nodosa, quoted Klemperer [6] as stating that the vascular lesion is not a necrotizing one but represents a hyaline or fibrinoid change. He also said that this may account for the fact that so many of these patients recover completely. "Whether they remain so, time

6. Klemperer, P., cited by Moschcowitz, E.: J. Mt. Sinai Hosp. **12**:1054-1063, 1946.

alone will decide." The relationship of this vascular change in the appendix to polyarteritis nodosa is of greatest importance since it concerns the ultimate fate of the patient.

Polyarteritis nodosa is known to involve the digestive tract in approximately 50 per cent of the cases. This rarely occurs without lesions being found in other systems. Although the presenting clinical features may be only those of involvement of an abdominal viscus, it is comparatively rare for these signs and symptoms to be only those of appendicitis.[7]

Gross and Friedberg [8] reported a case of a 33 year old man (case 3) in whom abdominal signs and symptoms predominated. The pathologic report of the appendix removed at operation recorded multiple foci of necrotizing arteritis. At necropsy the diagnoses were subacute polyarterities nodosa involving the arteries of the heart, kidneys, gallbladder, pancreas, liver, lungs, diaphragm, stomach, intestines and mesentery and rheumatic verrucous endocarditis. A photograph of the arteritic lesion of the appendix in this case reveals it to be similar to those described in this paper. Spiegel,[9] in a report of a number of cases of polyarteritis nodosa with a discussion of their clinical aspects, described 6 cases in which there were signs and symptoms of acute appendicitis or of an intra-abdominal suppurative condition. Two of the patients were subjected to operation, and the appendix revealed polyarteritis nodosa. She described the necropsy observations in the remaining 4 cases: In 3 the appendix had been removed at operation; it was stated to be normal in 2 cases, and in the third the diagnosis was subacute catarrhal appendicitis. The fourth patient had not been operated on, but no mention is made of the appendix. These patients were all females between the ages of 8 and 19. Regarding another case, not included in the series, Spiegel remarked that the lesions of the appendix were slight in comparison to the usual more "foudroyant" expression of the syndrome of polyarteritis nodosa and were of the type called necrotizing arteriolitis described by Plaut. In the latter condition there is absence of gross aneurysms and the extensive anatomic distribution seen in polyarteritis nodosa. She also made the statement that the histologic resemblance is important because it suggests that there may be an attenuated form of polyarteritis nodosa, and she expressed the belief that her cases 2 and 3, described as being instances of polyarteritis nodosa following appendectomy, may be examples of this arrested or attenuated form. The similarity of the appendical lesions described by Gross and Friedberg [8] and Spiegel [9]

7. Boyd, L. J.: Bull. New York M. Coll., Flower & Fifth Ave. Hosps. **4**:27-32, 1941.

8. Gross, L., and Friedberg, C. K.: Arch. Int. Med. **54**:170-198, 1934.

9. Spiegel, R.: Arch. Int. Med. **58**:993-1040, 1936.

as being present in cases of polyarteritis nodosa and those described by us suggests that we are dealing with a localized manifestation of polyarteritis nodosa.

Unfortunately, information concerning the subsequent clinical course of the 22 patients over a satisfactory period following appendectomy is unavailable; hence we are unable to state whether there has been a recurrence of the abdominal signs and symptoms. Several of the patients were reported as being well six months after the surgical procedure.

In recent years there has been a strong trend toward the view that polyarteritis nodosa is an allergic tissue reaction. Gerber,[10] by means of repeated intravenous injections of bacterial filtrates, produced among other changes a necrotizing arteritis limited to the kidney. Masugi and Isibasi [11] obtained diffusely distributed lesions of polyarteritis nodosa by sensitizing animals with various bacterial strains. Metz [12] reported in 1931 that he had produced lesions identical with those of periarteritis nodosa by sensitizing animals with foreign serum and the streptococcus. These experiments and the clinical observations suggest the possibility that the appendical lesion could be the result of a local allergic reaction to bacteria or their products.

CONCLUSION

Arteritis of the appendical arteries and arterioles is an occasional finding in an otherwise normal appendix of a patient who has the signs and symptoms of acute appendicitis.

The arterial lesion is possibly allergic in origin, representing either a focal allergic reaction to bacteria or bacterial products or a local manifestation of a generalized vascular lesion resembling polyarteritis nodosa.

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10. Gerber, I. E.: Arch. Path. **21**:776-796, 1936.

11. Masugi, M., and Isibasi, T.: Beitr. z. path. Anat. u. z. allg. Path. **96**:391-425, 1936.

12. Metz, W.: Beitr. z. path. Anat. u. z. allg. Path. **88**:17-36, 1931.

Case Reports

THROMBOSIS OF THE HEPATIC VEINS

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OCCLUSION of the hepatic veins is a clinicopathologic entity known as Chiari's syndrome. The condition was first described by Lambron in 1842, as cited by Budd [1] in 1846. The pathologic changes producing the syndrome were described by Chiari [2] in 1899. Because it is infrequently diagnosed during life, and because no successful method of treating it is known, a review of the literature and a report of a case are presented here in the hope of stimulating interest in the recognition and treatment of this syndrome.

INCIDENCE

Occlusion of the hepatic veins occurs with equal frequency in the two sexes and has been reported to occur in all age groups from 17 months to 70 years. It occurs most frequently between the ages of 20 and 40 years. In only 5 of 11,979 autopsies done at Stanford University [3] was thrombosis of hepatic veins encountered. According to Kelsey and Comfort,[4] the Mayo Clinic records from 1910 to 1939 showed only 20 cases of thrombosis of hepatic veins, and in 16 of these the thrombosis was an incidental finding at necropsy and had not produced recognizable symptoms.

CAUSATION

Armstrong and Carnes [3] have listed six causes for the syndrome. Kelsey and Comfort [4] classified the causes as primary or secondary. Primary occlusion is rare, but explanations for its occurrence are many and have been well summarized by Thompson and Turnbull [5] and Hutchinson and Simpson.[6] Among these explanations is the one originally advanced by Chiari, who thought the occlusion was due to primary inflammation of the veins, with or without thrombosis. A second hypothesis, supported by Thompson and Turnbull,[5] holds that the occlusion is due to primary thrombosis and that the inflammatory changes in the wall are secondary. A mechanical hypothesis was advanced by Kretz,[7] who

From the Department of Pathology, Montana Deaconess Hospital.

1. Budd, G.: Diseases of the Liver, Philadelphia, Lea & Blanchard, 1846, p. 152.
2. Chiari, H.: Beitr. z. path. Anat. u. z. allg. Path. **26**:1, 1899.
3. Armstrong, C. D., and Carnes, W. H.: Am. J. M. Sc. **208**:470, 1944.
4. Kelsey, M. P., and Comfort, M. W.: Arch. Int. Med. **75**:175, 1945.
5. Thompson, T., and Turnbull, H. M.: Quart. J. Med. **5**:277, 1912.
6. Hutchinson, R., and Simpson, S. L.: Arch. Dis. Childhood **5**:167, 1930.
7. Kretz, R.: Ergebn. d. allg. Path. u. path. Anat. **8**:498, 1902.

stated that the liver is more or less suspended by the hepatic veins and that stresses and strains on these veins result in formation of scar tissue which occlude the ostiums of the veins. Congenital defects have been proposed by Rosenblatt and Moor, as cited by Kelsey and Comfort,[4] and Hutchinson and Simpson.[6] These include failure of the hepatic veins and the inferior vena cava to unite, owing to fibrosis following fetal interstitial hepatitis, and obliteration of the vessels in this area in the same process which obliterates the ductus venosus. Secondary causes of occlusion of the hepatic veins may be divided into intrahepatic and extrahepatic. The intrahepatic causes may be inflammatory, neoplastic or cirrhotic processes, which by pressing on or narrowing the lumens predispose to thrombosis. Extrahepatic causes are numerous [8] and include trauma, perihepatitis, scars, thrombosis, neoplasms of the inferior vena cava, constrictive pericarditis, and diseases which cause multiple thromboses (i. e., primary polycythemia, or polycythemia vera, and thrombophlebitis migrans).

CLINICAL FORMS

Two clinical forms are described, acute and chronic. The acute form has a sudden onset with obscure abdominal pain, nausea, vomiting and shock. This is followed usually by a rapid accumulation of ascitic fluid, which is resistant to diuretics. The liver is enlarged and tender. There may or may not be a large spleen. Usually delirium and coma ensue, and death occurs within one to four weeks. The chronic form has a gradual onset with symptoms of indigestion, vague abdominal pain, ascites and hepatomegaly. There may be cyanosis, but rarely is there jaundice. Coma and death often occur in about six months, although the patient may live for several years. Death is usually due to hepatic insufficiency. However, there may be oliguria, acidosis and a state similar to the hepatorenal syndrome.[9] According to Hoover,[10] symptoms and signs may not occur until the occlusion of the hepatic vein is almost complete. Hepatic vein occlusion may be a severe complication of infection or of polycythemia vera (primary polycythemia).[8f]

The syndrome is rarely diagnosed during life. Thompson [11] found that 8 of the reported cases had been so diagnosed and reported 2 cases of his own. The enlargement of the liver and the ascites are more prominent in hepatic than in portal vein obstruction.[8f] The collateral circulation is cephalad in obstruction of the inferior vena cava, whereas it tends to be caudad in obstruction of the hepatic vein.[8d] In cirrhosis the liver is usually not enlarged and smooth, but is nodular, and the ascites does not

8. (*a*) Hess, A. F.: Am. J. M. Sc. **130**:1986, 1905. (*b*) Altschule, M. D., and White, G.: New England J. Med. **220**:1030, 1939. (*c*) Rolleston, H., and McNue, G. W.: Diseases of the Liver, Gallbladder, and Bile Ducts, ed. 3, London, The Macmillan Company, 1929. (*d*) Hollock, P., and others: Arch. Int. Med. **66**:50, 1940. (*e*) Norman, I. L., and Allen, E. V.: Am. Heart J., **13**:257, 1937. (*f*) Sohval, A. R.: Arch. Int. Med., **62**:925, 1938. (*g*) Baehrm, G., and Klemperer, P.: M. Clin. North America **14**:391, 1930.

9. Jacobson, V. C., and Goodpasture, E. W.: Arch. Int. Med. **22**:86, 1918. Kahn, S., and Spring, M.: Ann. Int. Med. **14**:1075, 1940. Wilensky, A. O.: Arch. Surg. **38**:625, 1939.

10. Hoover, C. F.: J. A. M. A. **74**:1753, 1920.

11. Thompson, M. D.: Arch. Int. Med. **80**:602, 1947.

occur as rapidly. Jaundice usually accompanies cirrhosis. Ascites due to cardiac failure can be ruled out by determinations of circulation time and readings of venous pressure. When associated with obstruction of the inferior vena cava the syndrome has been mistaken for constrictive pericarditis [8d]; however, fluoroscopic examination of the heart and kymographic studies rule out the cardiac lesion. The frank jaundice which occurs in acute hepatitis is not present in Chiari's syndrome. Splenic vein thrombosis produces pain in the left upper quadrant of the abdomen, and there is no associated hepatomegaly.

Abdominal pain, severer in the right upper quadrant, sometimes radiating to the back and shoulders, when accompanied with a rapid accumulation of ascitic fluid, resistant to diuretics, and with simultaneous enlargement of the liver, which is tender, as well as with edema of the lower extremities, should suggest obstruction of the hepatic vein.

PATHOLOGIC ASPECTS

The thrombosis occurs most frequently at the junction of the hepatic veins and the inferior vena cava. Its occurrence is probably due to eddying currents formed at the oblique-entering angle.[12] Inflammatory changes often occur in the inferior vena cava as well as in the hepatic veins, and it is thought that their proximity and eddying currents aid in spreading the infection from one vein to another.[13] The thrombosis may be partial, complete, or complete with recanalization.[14] The amount of collateral circulation depends on the speed of obstruction of the hepatic veins,[5] and the symptoms experienced by the patient depend on the ratio of collateral circulation to circulation lost by the obstruction.[6] The spleen may be enlarged.[6] The enlargement is due to engorgement. The organ is actually smaller than normal when the blood has been removed. Microscopically, there are central hepatic lobular necrosis and congestion of blood in this area, and there may be fibrotic replacement of the liver substance. Simonds and Callaway,[15] in 1932, and Simonds and Jergesen,[16] in 1935, experimentally produced acute obstruction of the hepatic veins in animals and studied the histologic changes that occurred in the liver.

No successful method of treatment has been offered. The patients all died shortly after omentopexy and other operations.[17]

REPORT OF A CASE

G. J., a 39 year old white married woman, entered the Montana Deaconess Hospital complaining of abdominal pain of two months' duration and rapid swelling of the abdomen of one week's duration. She stated that she began to have "gas on her stomach," with belching, about two months before she entered the hospital. This was accompanied by a feeling of general soreness throughout the entire abdomen. She felt quite irritable and "run down." She consulted a physician, who

12. Hoover.[10] Baehrm and Klemperer.[8e] Jacobson and Goodpasture.[9]

13. Rignon, R. H.: Bull. John Hopkins Hosp. **53**:162, 1933.

14. McAlpin, K. R., and Smith, K. E.: New York State J. Med. **38**:101, 1938. Hutchinson and Simpson.[8] Baehrm and Klemperer.[8e]

15. Simonds, J. P., and Callaway, J. W.: Am. J. Path. **8**:159, 1932.

16. Simonds, J. P., and Jergesen, F. H.: Arch. Path. **20**:571, 1935.

17. Dickinson, A. M.: Surgery **9**:567, 1941. Oppenheimer, B. S.: Tr. A. Am. Phys. **44**:338, 1929.

took roentgenograms of the gallbladder and told her she had gallstones. About one month after the onset of her first symptoms she began to have recurrent episodes of vomiting, followed by loss of consciousness of several minutes' duration. She also noted sharp epigastric pain which radiated around both sides of her back. She had one episode of rather severe pain of this nature which lasted for three days, after which she felt well for about two weeks. Then she had an exacerbation of the bloating and the aching. She lost 10 pounds (4.5 Kg.) in this six week period. Six days before entering the hospital she noticed the sudden development of generalized abdominal swelling. This continued and she began to have difficulty getting her

Fig. 1.—The orifices of the hepatic veins are closed except for a minute opening on the left. Two of the five slitlike openings in the inferior vena cava below the junction of the hepatic veins are seen.

breath because of the swelling. She frequently vomited after eating. She had constant dull abdominal pain. Systemic review revealed no other complaints.

The patient had undergone an appendectomy twenty years before. She had had three full term normal deliveries—six, five and two years previously. The family history was noncontributory.

Physical examination revealed blood pressure, 104 systolic and 75 diastolic; temperature, 98.6 F.; pulse rate, 100; respiratory rate, 20. The patient was a pale white woman, who appeared to be chronically ill. She complained of steady, gen-

eralized abdominal aching. Head, eyes, ears, nose, throat and cardiovascular-respiratory systems were essentially normal. The abdomen is stated to have been generally enlarged to about the size of a six to seven months' pregnancy. Shifting dulness was present. The abdomen was tense, and no masses could be palpated. The pelvis, the rectum and the extremities were normal to examination. An abdominal paracentesis was done and 7,700 cc. of straw-colored fluid obtained. Subsequent to the paracentesis the patient felt greatly relieved. There was tenderness to palpation over the entire abdomen, but this was most marked in the epigastrium. The liver was palpable 4 to 5 cm. below the right costal margin. The edge was smooth, firm and slightly rounded.

Hospital Course and Treatment.—The body temperature remained normal except for an occasional rise to 100 F. The pulse rate ranged between 80 and 100. The

Fig. 2.—Note the large thrombi in the veins.

respirations were normal. No abnormal stools were noted. She had an average oral intake of fluid of approximately 2,500 cc. The paracentesis wound continued to drain, but, in spite of this, fluid gradually accumulated in the abdomen. A second paracentesis was done on the eighth hospital day, and 4,500 cc. of straw-colored fluid was obtained. Cancerous cells were not found in the ascitic fluid. The erythrocyte count on admission was 4,950,000; the hemoglobin content 18 Gm.; the leukocyte count was 11,900; the differential showed a slight shift to the left. The blood urea nitrogen and blood chloride levels were normal. Total serum protein was 5.55 Gm., with 3.60 Gm. as albumin and 1.85 Gm. as globulin. The serologic tests gave negative results. On the tenth hospital day an exploratory laparotomy was performed. The liver was noted to be very large, smooth, firm and deep purple. Otherwise, the abdominal organs appeared normal. Two biopsy specimens were taken from the liver, and a subcutaneous button was installed below the umbilicus for

drainage of the ascitic fluid. The patient received 500 cc. of whole blood during the operation and left the operating room in good condition. Shortly after arriving in her room she went into shock. After a blood transfusion and administration of oxygen and stimulants, the blood pressure rose to 110 systolic and 70 diastolic. The blood pressure again became imperceptible on the fifth postoperative day and the patient went into coma. The laboratory studies done at this time showed an erythrocyte count of 6,250,000, a hemoglobin content of 21.4 Gm., a leukocyte count of 15,100 and an essentially normal differential count. Blood urea nitrogen and

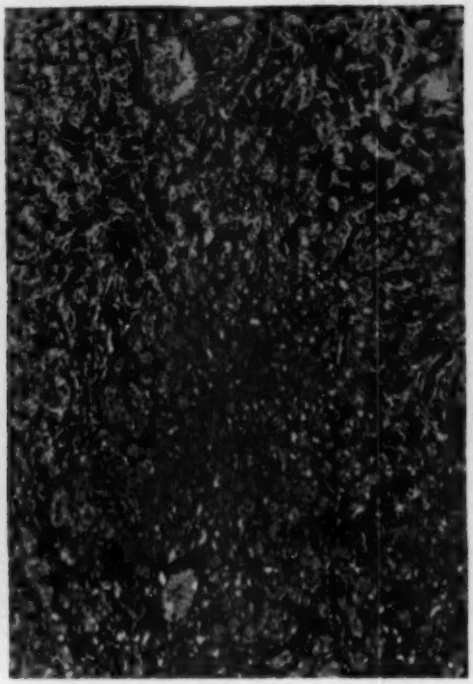


Fig. 3.—Note the dilated central vein and blood sinusoids in the lower part of the picture. The absence of hepatic cords in this area is also pronounced. Hepatic cords are seen in the upper portion of the picture, in the region of the triad.

blood chloride levels were normal. Biopsy of the liver showed definite pressure atrophy of liver cords extending from the central portion of the hepatic lobule toward the periphery. The central veins and blood sinusoids were dilated. There was deposition of brown pigment in the central portions of the lobules. Despite all efforts, the patient's condition continued to get worse; the pulse was weak and thready, respirations were irregular, and the blood pressure was unobtainable. This condition prevailed until the tenth postoperative day, when the patient died.

Autopsy.—The pertinent findings were (1) edema of the lower extremities, (2) ascites (2,500 cc.), (3) enlargement of the liver: The liver and the gallbladder together weighed 1,620 Gm. The surface of the liver was smooth. There were five small slitlike venous openings in the inferior vena cava as it passed the liver. Two of these apparently originated in the left lobe, and three in the right lobe. The largest measured 0.1 cm. in length. The ostium of the left hepatic vein (fig. 1) had one small opening, 0.1 cm. in diameter, and the ostium of the right hepatic vein was completely closed. The cut surface of the liver (fig. 2) was dark red. The hepatic veins were filled with dark red blood clot. The portal and splenic veins were only slightly dilated. The hepatic artery appeared to be normal. The inferior vena cava showed no gross changes throughout its entire course. Microscopically (fig. 3) the central veins and blood sinusoids were dilated. There was frank pressure

Fig. 4.—The section was taken at the junction of the right hepatic vein and the inferior vena cava. The inferior vena cava is cut transversely and is on the superior side of the fibrous band. The hepatic vein is cut longitudinally and is on the inferior side of the fibrous band.

atrophy of the liver cords extending from the central portion of each hepatic lobule toward the periphery. There was deposition of brown pigment in the centers of the lobules. There was slight increase of fibrous tissue about the triads, and many small bile ducts were present. The sections taken at the junction of the hepatic veins and the inferior vena cava (fig. 4) revealed a thick, dense fibrous tissue band occluding the hepatic vein openings into the vena cava. Both sides of this septum were covered with endothelium.

COMMENT

This case probably represents an acute form of Chiari's syndrome. It is interesting that the fibrous bands which occluded the ostiums of the hepatic veins except for a minute opening in the left had evidently been

sufficient to allow the patient to be symptom free until two months before death, when for some unknown reason symptoms of obstruction began to occur. The hemoconcentration noted in this case probably was due to dehydration rather than to primary polycythemia. A similar case with a fibrous band closing the ostiums of the hepatic veins is cited by Kelsey and Comfort.[4] Whether in our case the fibrous band was congenital or was on the basis of thrombophlebitis, it is impossible to state. No successful method of treatment has been offered for this condition. Simonds[18] produced the acute syndrome in animals by ligating the hepatic veins. Perhaps in cases of this syndrome an Eck fistula may offer a successful method of treatment.

SUMMARY

The literature on the incidence, causes, clinical forms and pathologic changes of Chiari's syndrome is reviewed.

A case of Chiari's syndrome is reported, in which in a 39 year old white woman a fibrous band occluded the ostiums of the hepatic veins except for an opening 0.1 cm. in diameter in the ostium of the left hepatic vein.

18. Simonds and Callaway.[15] Simonds and Jergesen.[16]

Notes and News

Registry of Hepatic Pathology.—A registry of hepatic pathology has been established, under the sponsorship of the American Gastroenterological Society, at the American Registry of Pathology, administered under the auspices of the National Research Council. Collections will be made of liver specimens obtained by biopsy or autopsy and accompanied with laboratory and clinical records. These specimens will be available for research. Material contributed will be reviewed by the Armed Forces Institute of Pathology, and an opinion concerning it will be furnished the contributor. Material should be submitted through a pathologist on forms obtained from the Institute; 1 stained and 5 unstained sections of liver biopsy specimens are requested, and 1 stained section and paraffin blocks or wet tissues of autopsy material. Specimens should be sent to the Director, Armed Forces Institute of Pathology, Registry of Hepatic Pathology, Washington 25, D. C.

Dr. Winternitz Retires.—Dr. Milton C. Winternitz, professor of pathology and for fifteen years dean of the Yale School of Medicine, New Haven, Conn., has retired after thirty-three years of teaching on the Yale faculty. He will continue as director of the Board of Scientific Advisers of the Jane Coffin Childs Memorial Fund for Medical Research, with offices in the Sterling Hall of Medicine.

International Congress of Clinical Pathology.—The International Congress of Clinical Pathology will meet in London, July 16-20, 1951. Members of the American Society of Clinical Pathologists will be sent a preliminary brochure. Other interested persons may secure this brochure after July 15 from the American Society of Clinical Pathologists, 1040-1232 West Michigan Street, Indianapolis 7, Ind.

Dr. Wolbach Honored.—The Howard Taylor Ricketts Medal, endowed last year by Mrs. H. T. Ricketts, was awarded to Dr. S. Burt Wolbach, Harvard University professor emeritus of pathology, May 8, at the Albert Merrit Billings Hospital, Chicago. Dr. Wolbach made some of the earliest studies on the pathology of Rocky Mountain spotted fever and typhus. He is also known for his contributions to the knowledge of vitamin A and C deficiency diseases. In 1920 he served as director on the League of Red Cross Societies, Research Committee on Typhus Fever to Poland. After presentation of the medal, Dr. Wolbach spoke on rickettsial diseases.

Twenty-Sixth Ludvig Hektoen Lecture of the Frank Billings Foundation.—Jesse E. Edwards, assistant professor of pathologic anatomy, Mayo Foundation, University of Minnesota, gave the twenty-sixth Ludvig Hektoen Lecture of the Frank Billings Foundation of the Chicago Institute of Medicine on May 26. His subject was "Structural Changes of the Pulmonary Vascular Bed and Their Functional Significance in Congenital Cardiac Disease."

Books Received

DAS MENSCHLICHE KNOCHENMARK—SEINE ANATOMIE, PHYSIOLOGIE UND PATHOLOGIE NACH ERGEBNISSEN DER INTRAVITALEN MARKPUNKTION. By Prof. Dr. Med. Karl Rohr, University of Zurich. Second edition. Pp. 404, with 143 illustrations. Price 47.50 German marks. George Thieme, Diemershaldenstrasse 47, Stuttgart-O, 1949.

The author concluded the preface to the first edition with the regret that his teacher Prof. Otto Naegeli, one of the founders of modern clinical hematology, died before this book could be dedicated to him. In thoroughness and breadth of vision there is much in this volume that reminds one of Naegeli's remarkable book "Blutkrankheiten und Blutdiagnostic," the fifth edition of which appeared in 1931.

The monograph is divided into a general and a special part. The former begins with a brief historical review of marrow puncture, followed by an excellent discussion of the technic of the operation, of the preparation of smears, the counting of cells and the limitations of the procedure. The embryologic aspects of the subject and cytogenesis, the anatomy of marrow, the special cytology of myeloid, erythroid and reticulohistiocytic elements, of megakaryocytes and their products, of karyokinesis and karyorrhexis, and other morphologic data are presented in great detail. Of special value to the pathologic anatomist will be the chapter on postmortem changes.

Much emphasis is placed on physiology, including central nervous, vegetative, pharmacologic and hormonal regulatory mechanisms, interrelations with the lymphatic system (hypersplenism and hyposplenism), regulation of release of marrow cells, origin of abnormal myeloid cells found in peripheral blood, effects of radiation, marrow cultures and use of the sternum for transfusions. A chapter of the general pathology of marrow is given to such topics as hypoplasia and aplasia, hyperplasia and neoplasia, inflammation and the problem of leukemia. Part one concludes with an instructive fourteen point summary.

Part two deals with the special pathology of erythropoiesis, leukopoiesis, thrombopoiesis, lymphopoiesis, panmyelosis (the marrow equivalent of pancytopenias), the reticulohistiocytic system and the neoplastic and infectious diseases of marrow.

The volume has increased by 118 pages since the first edition. The treatment of the subject is essentially similar. However, whereas ten years ago attention was focused on such subjects as cytology, pernicious anemia and granulocytopenia, more recently the reticulohistiocytic system, the hemolytic and aplastic anemias and the leukemias have claimed more interest. This trend is reflected in the shift of emphasis in the book.

Rohr has definite and, among them, original ideas about several problems of marrow pathology. He puts them forth clearly and without hesitation, always against the backdrop of other views. The reader will be enriched and stimulated by this book even if he does not agree with some of its ideas.

The illustrations, many in color, are of high quality.

The bibliography includes a list of books on hematology, another of monographs on marrow puncture, and finally a 33 page, well selected list of references, in which American publications are well represented.

There is no question but that this is the most complete and comprehensive monograph on this subject. It can be recommended to all interested in bone marrow.

AN ATLAS OF THE BLOOD AND BONE MARROW. By R. Philip Custer, M.D., director, Laboratories of the Presbyterian Hospital, Philadelphia; assistant professor of pathology, University of Pennsylvania School of Medicine; consultant to the Armed Forces Institute of Pathology. Pp. 321, with 285 illustrations, 42 in color. Price $15. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1949.

The need for a concise photographic reference to the pathologic anatomy of peripheral blood and bone marrow is partially fulfilled by this book. It is not an atlas in the didactic sense, but is more practically aimed at correlating clinical disease states with their blood and marrow changes through a pertinent text and abundant photomicrographs.

The material is presented in two sections. The first is designated "the hemolytopoietic system," after Krumbhaar, and comprises three chapters. The intial chapter consists almost wholly of a reprint of the first report of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs, sponsored by the American Medical Association and the American Society of Clinical Pathologists (*Am. J. Clin. Path.* **18**:443, 1948). The nomenclature recommended in this report is used thereafter throughout the book, with the exception of that relating to the red cell series. Chapter 2 and 3 present, respectively, succinct discussions of embryogenesis and postnatal hemopoiesis, employing a practical compromise schema of blood cell origin.

The second section is devoted to diseases of the blood and marrow, and includes successive chapters on deficiency anemias; aplastic and hypoplastic anemias; displacement of bone marrow; hypersplenism; hemolytic anemias; hemorrhagic states; effects of physical and chemical agents; leukocytosis, leukemoid reactions, and leukopenia; and polycythemia. In a concluding chapter on technics, that of sternal puncture and that of biopsy are adequately discussed, and methods of spinous process and iliac crest puncture are included.

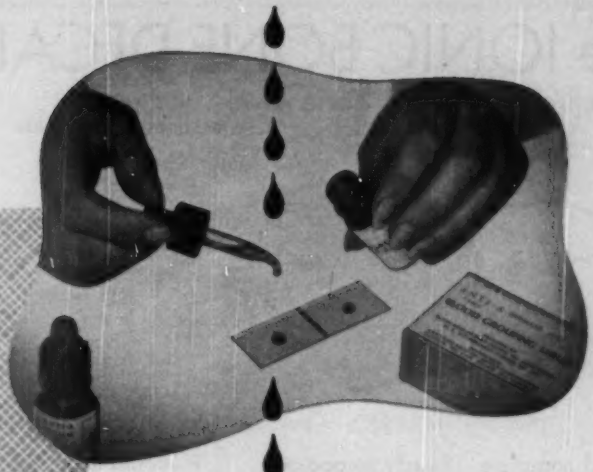
The author's style is refreshingly concise and lucid. The text is patterned on a brief discussion of the clinical findings and the course of a hematologic disease, the significant laboratory data concerning peripheral blood and marrow, and the established forms of treatment. Attention is properly drawn to clinical findings and adjunct laboratory tests to obviate undue diagnostic emphasis on the blood picture alone. There are critical comments on recent therapeutic agents: folic acid, vitamin B_{12}, the folic acid antagonists and radioactive phosphorus. Several useful tables are provided, including normal and abnormal blood values, classification and differential diagnosis of blood diseases, and etiologic agents in hemolytic anemias and the hemorrhagic states. References to the literature are restricted to a few pertinent articles and are placed at the bottom of appropriate pages.

The work is profusely illustrated (238 black and white and 42 colored illustrations and 9 other figures). Accompanying legends frequently include salient clinical information which heightens their interpretative value. Magnification of photographs are consistently indicated. While many are superb photomicrographs at high magnification (as great as × 2,280), some have been enlarged beyond good definition and orienting perspective. The author has placed great emphasis on marrow sections, illustrations of which outnumber those of peripheral blood and marrow smears. This valuable correlative feature will appeal to the tissue pathologist.

However, it is felt that closer editing of repetitive photographs (7 on multiple myeloma, 5 on sickle cell anemia and 5 on aplastic anemia due to quinacrine hydrochloride [atabrine dihydrochloride® mepabrine hydrochloride]), and reduction in page size of many others would provide space for additional portrayal of smear cytology, and might increase the usefulness of the book for the clinical pathologist and the hematologist whose duties rarely bring them into contact with marrow biopsies.

The text is printed in pleasing two column format and in readable type. There is an adequate general index. Few typographic errors are detected. Incorrect figure references appear on pages 196 and 198, and the author reference in connection with figure 156 is in error.

This book forms a useful contribution to the rapidly growing hematologic literature, and a handsome supplement to the more definitive textbooks on blood diseases. It should be in the hands of all pathologists, and available to all other physicians interested in the broad field of hematology.



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